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Predictive Modeling of Fate and Transport of Three Prevalent Contaminants in Midwest Agroecosystem Surface Waters: Nitrate-N, Atrazine, and *Escherichia coli*

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PREDICTIVE MODELING OF FATE AND TRANSPORT OF THREE PREVALENT CONTAMINANTS IN MIDWEST AGROECOSYSTEM SURFACE WATERS: NITRATE-N, ATRAZINE, AND *ESCHERICHIA COLI*

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The majority of streams and rivers in the United States (U.S.) are ecologically impaired, or threatened by anthropogenic stressors. Recent reports have found atrazine in drinking water to be associated with increased birth defects and incidences of Non-Hodgkin's Lymphoma, with higher levels of significance from exposure to both atrazine and nitrate-N. In contrast, recent illnesses from *E. coli* contaminating vegetables that originated from irrigation water has increased awareness of identifying sources of *E. coli* entering irrigation reservoirs.

Methods to accurately predict atrazine and *E. coli* occurrence and potential sources in waterways continue to limit the identifying appropriate and effective prevention and treatment practices. Therefore, the primary objectives of this study were to: 1) Identify watersheds across Nebraska that were at risk for exceeding nitrate-N and atrazine maximum contaminant limits (MCLs) in surface water, 2) Determine the specific times of greatest risk for exposure to atrazine throughout the year, 3) Determine the load of *E. coli* during storm events in a hydrologic controlled stream situated adjacent to a livestock

grazing operations and centered in the fly zone for avian migration in the Midwest, and 4) Identify trends between *E. coli* concentrations, grazing rotations, and avian migrations patterns.

Findings from objectives 1 and 2 of this project identified impairments for both nitrate-N and atrazine in the surface water during the early growing season in the southeastern region of Nebraska. Objectives 3 and 4 required a complex combination of bovine density and waterfowl migration patterns to evaluate the impact of *E. coli* concentrations in stream water, with the downstream reservoir had exceedance probabilities above the EPA freshwater criteria >85% of the growing season following rainfall events. Further, methodology developed in this project has the potential for application in regions with higher dependency on surface water to determine agrochemical and *E. coli* load influxes from upstream regions, evaluate other surface water contaminants in surface and/or groundwater, and implement best management practices.

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CHAPTER 1: LITERATURE REVIEW

PREDICTIVE MODELING OF FATE AND TRANSPORT OF THREE PREVALENT CONTAMINANTS IN MIDWEST AGROECOSYSTEM SURFACE WATERS: NITRATE-N, ATRAZINE, AND *ESCHERICHIA COLI*

Overall

Majority of streams and rivers in the United States (U.S.) are ecologically impaired, or threatened by anthropogenic stressors (Cardinale et al., 2012). Specifically, water is often threatened by the increasing use of pesticides to prevent crop damage (Vorosmarty et al., 2005) and *Escherichia coli* contributions from animal operations adjacent to streams (Wilkinson et al., 2011) and avian presence in waterways (Pendergrass et al., 2015). These stressors have led to increased costs for water treatment (Velten et al., 2007, Rompre et al., 2002), especially in states dependent on surface water. The use of pesticides has also resulted in the loss of biodiversity and ecosystem services (Vorosmarty et al., 2010). Further, the United States Department of the Interior estimated that 80 percent of the damage inflicted on riparian river systems in the arid western U.S. has been caused by cattle grazing operations (USDI, 1994). Therefore, the work presented in this thesis will focus on these specific water quality stressors in agroecosystems.

Atrazine Occurrence in Agroecosystems

Farmers of the Midwestern United States did not adopt the use of herbicides until the 1950's. Before that, farmers relied on tillage, hand hoeing, and crop rotation to reduce the loss of yield from weeds (Mannion, 1995). Atrazine was first developed in 1952 by the Geigy Chemical Company of Basel, Switzerland (now Syngenta). It was patented in 1958 and was registered for commercial uses in the United States by 1959 (Cripps and Roberts, 1978). Since then, atrazine has been a major herbicide used throughout the world due to its effectiveness at controlling grassy and broadleaf weeds, and its low cost. Annual atrazine application in the U.S. ranges from to 27 to 36 million kilograms (60-80 million pounds), 85 percent being applied for agricultural purposes (USEPA, 2003). Figure 1.1 shows the low-end estimates for agricultural atrazine use across the U.S. for 2016.

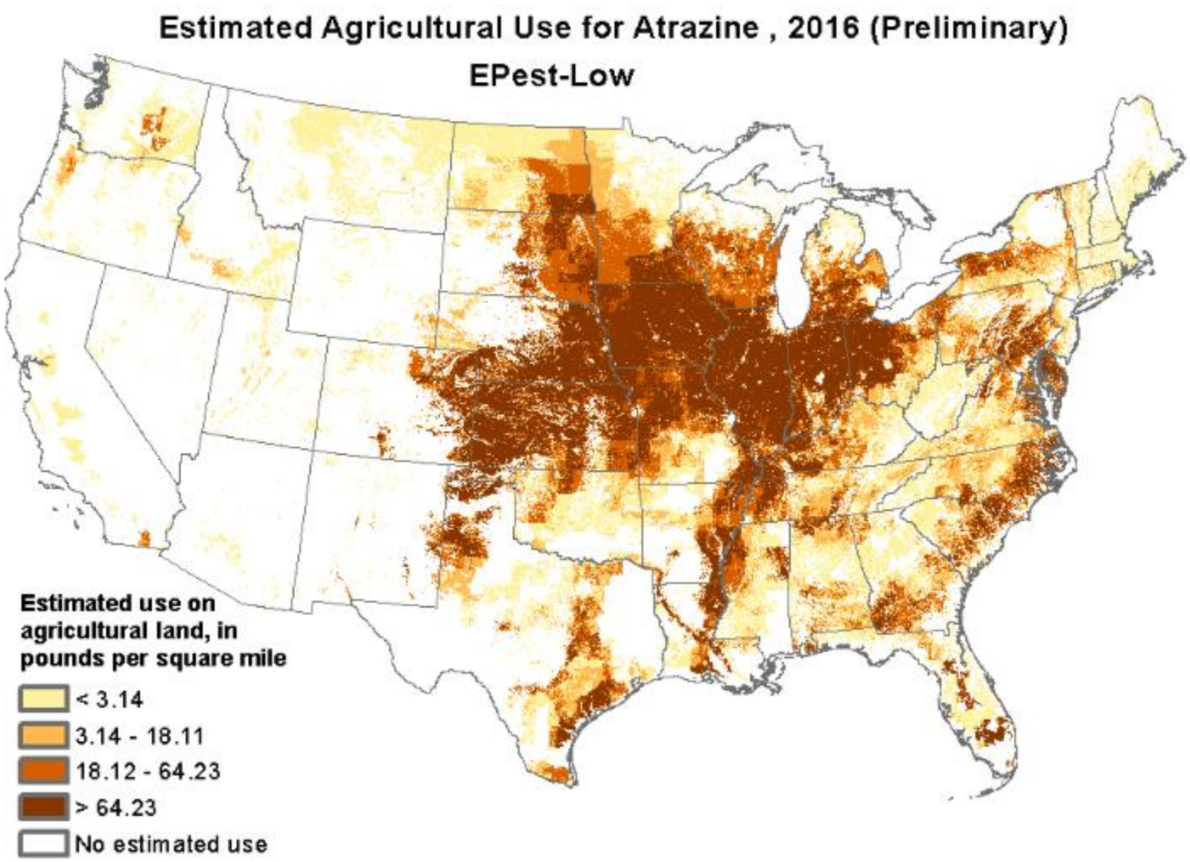

***Figure 1.1**: Estimated agricultural use of atrazine across the United States for 2016, retrieved from United States Geological Survey, 2016.*

Atrazine Nature

Atrazine uptake is primarily through the roots of plants (ATSDR, 2003). Atrazine works by blocking the electron transport mechanism in chloroplast's photosystem II complex to prevent the plants from producing energy and fixing carbon dioxide. The plants die by desiccation once the membrane is damaged from a chain reaction of lipid peroxidation (Brassard et al., 2003).

Atrazine is a highly mobile chemical compound due to its high water solubility of 33 mg/L (Mudhoo and Garg, 2011; ATSDR, 2003; Mackay et al., 1997). Further, atrazine's low vapor pressure (2.89 x 10^{-7} mm at 25°C) and Henry's law constant (2.48 x 10^{-9} atm m^3/mol) hinders it from volatilizing from surface water (Cripps and Roberts, 1978). The low rate of volatilization and low reactivity prevents atrazine from leaving water results in atrazine often being detected in surface and ground water throughout the Midwest.

Atrazine's frequency in surface water bodies is due to its mobility through the soil, intense usage, and moderate persistence (Jayachandran et al., 1994). Atrazine's presence in the Mississippi River and its tributaries have been extensively researched because of the potentially adverse ecological and human health concerns (Thurman et al., 1991; Battaglin et al., 2003; Kalkhoff et al., 2003; Scribner et al., 2005). Gilliom et al. (2006) collected data from agricultural streams across the United States from 1992-2001 and found atrazine in 85 percent of the samples collected. Detections of atrazine were even higher (98 percent) in post-application samples collected in Midwestern streams during the 1990's (Scribner et al., 2005).

Atrazine has a comparatively longer half-life in water than other herbicides at about 6 months (ATSDR, 2003). The same trend applies to the soil half-life of atrazine which is about 140-150 days, before degrading to deethylatrazine (DEA) and other degradates (Farrugia et al., 2016). The main risk of atrazine exposure to the aquatic ecosystem is the moderately to slightly toxic nature to many fish species, and its slightly less toxic nature to aquatic invertebrates (Brassard et al., 2003). Algae and aquatic vascular plans are also susceptible to atrazine's herbicidal effects.

Atrazine Transport

Atrazine can be applied pre-emergent to crops or after they emerged from the soil (ATSDR, 2003). Its application tends to be before weeds emerge during the months of highest precipitation events in the Midwestern United States (Thurman et al., 1991). This, along with the fact that it has a low water solubility and a six month half-life in water, causes a pulse of atrazine concentration in agricultural streams during the late spring months. This phenomenon is known as a "spring flush", where a seasonal pulse of herbicides, especially atrazine, occur after precipitation events following pre-planting application of herbicides (Stoeckel et al., 2012). The spring flush is a major factor in transporting atrazine from the applied cropland to surface water bodies, causing atrazine concentrations to rise (Thurman et al., 1991; Gilliom et al., 2006).

Atrazine Health Concerns

The United States Environmental Protection Agency (USEPA) also started conducting studies, including a special review in 1994 titled, "Atrazine, Simazine and Cyanazine; Notice of Initiation of Special Review." This study primarily focused on human health concerns and the potential effect atrazine may have on non-target terrestrial and aquatic biota (USEPA, 1994). The concerns for human health arise because of the endocrine disruption caused by exposure to atrazine (Forgacs et al., 2012).

A study conducted by Rhoades et al. (2013) analyzed the increased risk of Non-Hodgkin Lymphoma (NHL) from exposure to nitrate and atrazine in drinking water in the state of Nebraska. This study collected water quality data and weighted it by the contribution of the wells and the proximity of residence to the water supply. The collected data was analyzed using a logistic regression to determine 95 percent confidence intervals (CI) and odds ratios (OR). No association of a higher risk of NHL with exposure to either nitrate or atrazine alone was reported. However, dual exposure to both contaminants, elevated the risk of NHL (OR, 2.5; CI,1.0-6.2). The phenomenon was believed to be due to the *in vivo* formation of the nitrosamine *N*-nitrosoatrazine (NNAT) and its subsequent metabolism (Ward et al., 2005; Cova et al., 1996). Nitrosamines have a variety of forms; many of them known carcinogens (Krull et al., 1980). When humans ingest nitrate, it reduces to nitrite in the stomach and then allows secondary amines to be nitrosated due to the acidic conditions (Mirvish, 1975; Wolfe et al., 1976; Loeppky, 1994; Brambilla et al., 2009). Atrazine is a secondary amine and, when present, nitrosates to form NNAT, which has been linked to a higher chance of chromosomal abnormalities in lymphocytes *in vitro* at low doses (Meisner et al., 1993).

A study conducted by Stayner et al. (2016) analyzed the risk of preterm delivery (PTD,< 37 weeks), very preterm delivery (VPTD,< 32 weeks), low birth weight (LBW, < 2.5 kg among infants born at term), and very low birth weight (VLBW,< 1.5 kg) in four Midwestern states from exposure to atrazine and nitrate, separately and together. This study was one of the largest of its kind that explored the effects of agrichemical exposures through drinking water and the outcomes of births. Data obtained from 134,258 births in 2008 from 46 counties with public water systems that were a part of the U.S. EPA atrazine monitoring program (AMP). Data was obtained from each of the four states to estimate rates of PTD, VPTD, LBW, and VLBW. These rates were linked with the local monthly concentrations of atrazine and nitrate in finished drinking water. These variables were fitted using a multivariable negative binomial model to determine a correlation between adverse birth outcomes and the exposure of these two pollutants. A restriction was put on the data for counties that had low percentages (10 percent or 20 percent) of private drinking wells being used to prevent exposure misclassification. The results of this study produced a linear exposure-response relationship between the risk of PTD and VPTD and the local concentrations of atrazine in drinking water in counties with less than 10 percent use of private drinking wells during the subject's prenatal period. The correlation was particularly strong for exposure of atrazine between 4 to 6 months prior to birth for PTD, and for VPTD following exposure 0 to 3 months prior to birth. For nitrate exposure, there was also evidence that suggested a linear exposure-response relationship for the risk of VPTD, VLBW and exposure to nitrate in drinking water. The analysis of the VPTD data was restricted to <20 percent private well use and a highly significant (p=0.007) correlation was reported. A somewhat significant relationship (p=0.08) was determined between the VPTD and nitrate restricted to <20 percent private well use over 0 to 3 months before birth. These results for VPTD became even more significant (p=0.001) when atrazine and nitrate were included in the model.

Further, atrazine has been found to lower serum, testicular testosterone, and leutenizing hormone levels in rats (Stoker et al., 2000). Another study reported male frogs exposed to atrazine in water undergo feminization (Hayes et al., 2010). Lastly, two epidemiological studies examined the adverse potential effects on human reproduction, and birth outcomes from exposure to atrazine, and nitrate simultaneously.

Modeling Atrazine

Modeling of atrazine and other pesticides is vital to further our understanding of human exposure, as well as mitigating their dangers. Modeling enables development of predictive "hot spots" using spatial distribution analysis of atrazine and nitrate in surface water bodies across Nebraska.

A study conducted by Guardo A., and A. Finizio (2017) analyzed the spatial distribution of glyphosate in Lombardy, Italy. The researchers in this study used data from surface water monitoring stations, statistically analyzed their data using the software R, and correlated it with GIS to determine "high risk" areas. The procedure of this study contained two phases to address the environmental risk of pesticide residues. The data that was used in this study was available through either the Drinking Water Directive (Directive 98/83/EC) and the Water Framework Directive and the indicator for the areas that were at "high risk" were for glyphosate concentrations that surpassed the maximum acceptable levels, which were established by the Environmental Quality Standards (EQS). The first phase was the acquisition of data from the monitoring stations and the statistical analysis of the data, calculating for the 95th percentile of the Measured Environmental Concentrations ($MEC_{95th\ percentile}$). Once they determined the $MEC_{95th\ percentile}$ for each monitoring station, the second phase involved assigning a ratio (I_{EQS}^{95perc}) to determine the level of risk for these waterbodies. The ratio was the $MEC_{95th\ percentile}$ over the EQS and the corresponding range of ratios were divided into five classes. To determine the overall trend of the contamination of the waterbodies, an annual average index was determined for each monitoring station ($\overline{I_{EQS}^{95perc}}$). If $\overline{I_{EQS}^{95perc}}$ was less than 0.8, then the area was considered generally safe. If the $\overline{I_{EQS}^{95perc}}$ was in between 0.8 and 1, then it was considered to be at low risk. Areas were determined to be at risk if the $\overline{I_{EQS}^{95perc}}$ was in between 1 and 2 and at high risk if it was above 2. The study then classified these trends and correlated them with land use, to attempt to identify possible sources of glyphosate contamination. They then developed a method to mitigate the risk of surface water contamination using the trends observed from the data, GIS software, and the expert analysis of risk managers.

Another study conducted by Mahler et al. (2017) analyzed the similarities and differences between glyphosate, a common herbicide used in non-agricultural settings, and atrazine in occurrence in small Midwestern streams within the United States. The study used 100 total sites on shallow streams across the "corn belt" of the Midwestern United States for sample collection through collaborations between the U.S. Geological Survey (USGS), the Midwest Stream Quality Assessment (MSQA), the USEPA, and the National Rivers and Streams Assessment (NRSA). The USEPA selected 50 random sites to sample based off of the NRSA probabilistic design, and the remaining sites were selected to target urban land use (12 sites) and create a gradient in intensity of agricultural land use (38 sites). The study collected 12 weekly samples for every MSQA site from May 6 – August 9, 2013, with the expectation of two 2-week periods where only one sample was collected. In addition to the weekly sampling regime, the study also collected water samples using a more time-intensive method of every 2 days in a subset of 8 sites located in Missouri from May 15 – July 23. The atrazine concentrations were measured by enzyme-linked immunosorbent assay (ELISA) at the USGS Texas Water Science Center (TxWSC) for the 2-day samples with method reporting levels (MRL) for atrazine of 0.1 μg/L. The weekly samples for atrazine were analyzed at the USGS National Water Quality Laboratory (NQWL) by liquid chromatography tandem mass spectrometry (LC – MS/MS) (Sandstrom et al., 2015). The LC – MS/MS analysis was able to determine the concentration of atrazine and its degradate deethylatrazine (DEA) from the water samples. The MRL of atrazine and DEA were 0.005 µg/L and 0.011 µg/L, respectively. The statistical analysis for this study utilized the software Statistica v. 12, and the nonparametric Mann-Whitney U test evaluated the differences between populations. The nonparametric Kendall's tau correlation was used to determine correlations and trends using a significance level of $p \leq 0.05$. Maximum atrazine concentrations correlated with the soil K factor, organic matter content, permeability, and restrictive layer along with the base-flow index. Atrazine was detected more frequently and with higher concentrations in agricultural streams than urban streams. The first flush mechanism in late May was a vital determinant of the timing of peak atrazine concentrations in the streams sampled. Further, the weekly samples did not capture the peak concentrations found in the 2-day sampling methods, indicating the vital importance of more frequent sampling required to identify the spikes of concentrations of atrazine in surface water bodies. Finally, the maximum 21-day average atrazine concentrations were higher than the concentrations reported to affect fish health and reproduction (0.5 µg/L) at 75 percent of the sites (Papoulias et al., 2014; Tillitt et al., 2010), and the concentrations exceeded the maximum contaminant level (MCL) of 3 µg/L in 8 percent of the samples collected weekly.

E. coli Occurrence in the Agroecosystems

Escherichia coli is a bacterium found in the intestines of both people and warm-blooded animals. Therefore, it is used as an indicator of fecal contamination and the likelihood of pathogens present in water bodies. While most strains of *E. coli* are harmless, other strains such as *E. coli* O157:H7 can result in intestinal infections, dehydration, kidney failure, and death. Approximately $600 million were spent annually on medical expenses related to *E. coli* O157:H7 contamination, not including the other cases of exposure to non-O157:H7 strains of *E. coli* (Scharff, 2012). In 2010 alone, there were 63,153 cases, 2,138 hospital admittances, and 20 deaths form *E. coli* O157:H7 contamination. Children and the elderly are especially vulnerable to *E. coli* exposure from the complication of hemolytic uremic syndrome. This disease occurs in 2-7 percent of infections and can be extremely hazardous to humans due to the effects of the disease on red blood cells, leads to kidney failure. Hemolytic uremic syndrome is the main cause of acute kidney failure in children in the U.S. and the main culprit is exposure to *E. coli* O157:H7. This disease is considered a life-threatening condition that should be treated in an intensive care unit, where the death rate is 3-5 percent (USEPA, 2016). The two primary methods of *E. coli* O157:H7 transmission are through food and water. Over the last six years reservoir monitoring conducted by the Nebraska Department of Water Quality of 52 reservoirs across Nebraska reported reservoir closures for 335 weeks due to *E. coli* exceedance values (Figure 1.2). The most frequent location for water contamination occurs in runoff farms from manure applications, irrigation waters, and/or interactions with waterfowl. Further, *E. coli* continues to contaminate reservoirs in both agricultural and urban aquatic ecosystems, which results in further food security and health implications (Soller et al., 2010; Efting et al., 2011).

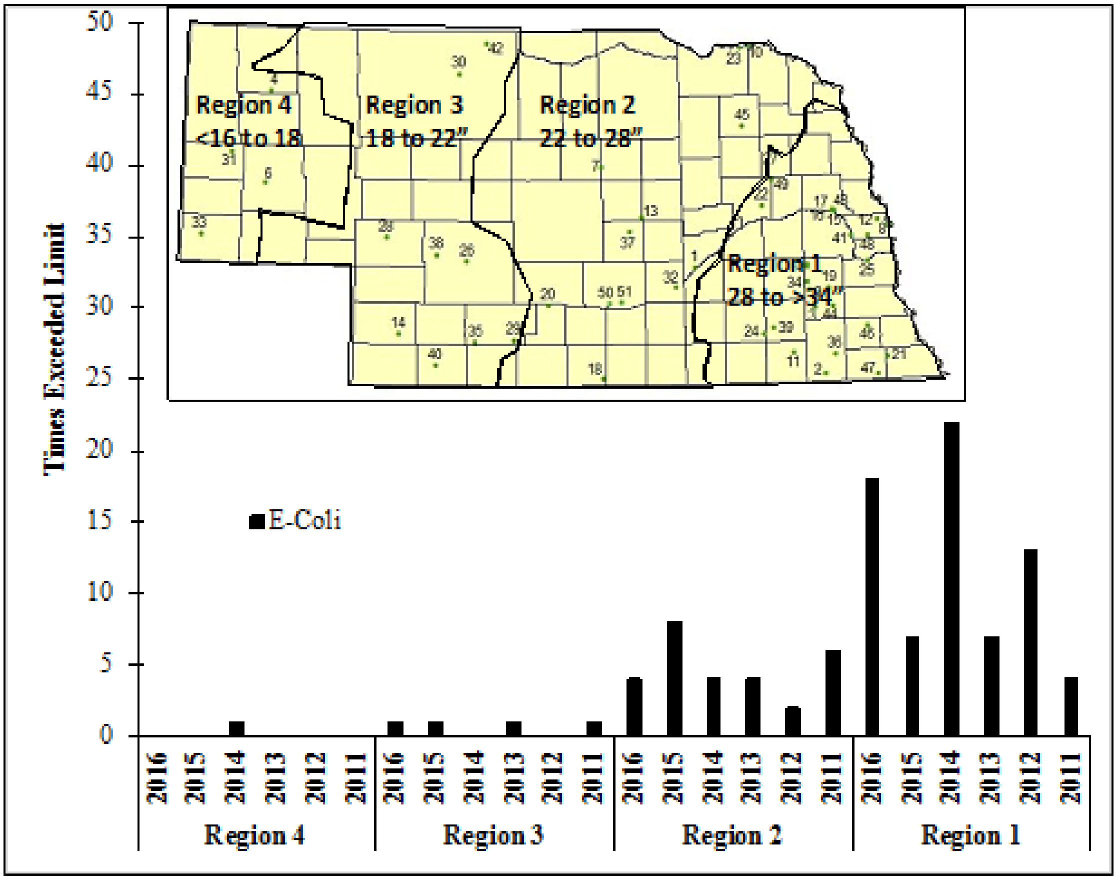

Figure 1.2: Incidences *E. coli* exceeded water quality standard yearly in 52 evaluated reservoirs by the Nebraska Department of Environmental Quality (NDEQ, 2017) (bar graph) and the precipitation regions of Nebraska (inset).

E. coli Transport

In agricultural settings, fecal bacteria and pathogens can contaminate surface water from non-point sources. These non-point sources can be the fertilizing of fields with manure and even by the direct addition of manure from grazing livestock (Gagliardi and Karns, 2000). The risk for surface water from run-off from agricultural activities has been generally accepted and understood because surface water is contaminated more frequently than groundwater. Groundwater is often considered relatively free of pathogen contamination due to the natural filtration the vadose zone provides before the microbes reach the water table (Rosen, 2000). Waterborne *E. coli* concentrations have been reported to show higher levels in areas below cattle grazing operations, compared to areas experiencing little to no human or cattle activity (Derlet et al., 2012).

Livestock grazing is frequently identified as a contributor of fecal coliforms, which has resulted in required measures by the United States government to reduce *E. coli* occurrences to improve surface water quality (TCEQ, 2007, 2008). Several studies that have shown a direct relationship between livestock grazing and an increase in *E. coli* concentrations in runoff and surface water bodies, resulting from either direct deposition of fecal matter, or subsurface and surface flow (Doran and Linn, 1979; Doran et al., 1981; Gary et al., 1983; Tiedemann et al., 1987; USEPA, 2001 Donnison et al., 2004). Surface runoff is the main method for the transport of *E. coli* into streams (Collins et al., 2005). Therefore, best management practices (BMPs) (e.g, vegetated filter strips; wetlands) have been recommended for grazing operations to reduce the occurrence of impaired streams by contamination by *E. coli* and other fecal coliform bacteria (Wagner et al., 2012).

Factors Affecting *E. coli*

Four major factors affect the probability and magnitude of *E. coli* entering waterbodies within a catchment. These factors include land use, climate, topography, and hydrology. The land use factor has a significant impact on the magnitude of the *E. coli* load that is entering the catchment. Climate plays a factor in the movement and inactivation of *E. coli* from precipitation that can lead to runoff and infiltration. Topographical factors of the landscape, including the subsurface medium, influence the movement and subsequent natural filtration of *E. coli* and waterborne pathogens. Hydrological factors consist of the direction of water movement and storage within a catchment. Infiltration, overland flow, and other routes of flowing water have a considerable impact on the movement and storage of pathogens (Rosen, 2000; Mawdsley et al., 1995).

The contamination of surface waters from *E. coli* and other fecal bacteria is a function of the characteristics of the fecal deposition site, size and quantity of livestock, locations of the livestock, livestock fecal deposits in relation to distance from waterbodies, and survival of bacteria from the time of deposition and surface runoff events (Larsen et al., 1994). However, recent findings have indicated waterfowl populations may significantly affect *E. coli* exceedance occurrences. To date little is known for predicting *E. coli* occurrence in waterways, which leads to challenges for identifying appropriate and effective preventative and treatment practices and water quality improvements (De Brauwere et al., 2014; Lothrop et al., 2018). Further, the ubiquitous occurrences of *E. coli* exceedances throughout the United States, particularly in water-limited areas such as the Midwest, distinguishes the urgency of identifying fate and transport patterns of *E. coli* in waterways. Therefore, a better understanding of the fate and transport of *E. coli* in agroecosystems would improve recommendations for monitoring practices and best management practices (BMP) for water quality improvements those waterbodies.

Overall Objectives

Based on the potential health and ecological implications of atrazine, NO_3-N, and *E. coli* being present in surface water, further investigation is required to identify “hot” times and “hot” spots in in the Midwest along with primary contributors. Therefore, the objectives of this project were:

1. Identify watersheds across Nebraska that were at risk for exceeding nitrate-N and atrazine maximum contaminant limits (MCLs) in surface water (Chapter 2);
2. Determine the specific times in the year where risks were greatest (Chapter 2);
3. Determine the load of *E. coli* during and following storm events at a continuous rotational livestock grazing operation in central Nebraska (Chapter 3)
4. Identify trends between *E. coli* concentrations in water, cattle grazing rotations, and avian migration patterns (Chapter 3).

CHAPTER 2: MITIGATING THE RISK OF ATRAZINE EXPOSURE: IDENTIFYING HOT SPOTS AND HOT TIMES IN SURFACE WATERS ACROSS NEBRASKA, USA

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Abstract

Atrazine, one of the most widely used herbicides in the world, threatens human health along with terrestrial and aquatic biota. Recent reports have found atrazine in drinking water to be associated with increased birth defects and incidences of Non-Hodgkin's Lymphoma, with higher levels of significance from exposure to both atrazine and nitrate-N. The Midwest region of the United States, which includes Nebraska, is one of the leading regions for high nitrate-N concentrations and agrochemicals, including atrazine, in surface and groundwater. Therefore, the objectives of this study were to: 1.) Identify watersheds across Nebraska that were at risk for exceeding nitrate-N and atrazine maximum contaminant limits (MCLs) in surface water, and 2.) Determine the specific times of greatest risk for exposure throughout the year. The study utilized a risk factor assessment for atrazine and nitrate-N concentrations to determine watersheds with the greatest risk for surface water impairments. Factors were then analyzed using Geographic Information System (GIS) software to identify areas of high risk. Impairments for both nitrate-N and atrazine in the surface water were found predominately during the early growing season in the southeastern region of Nebraska, in watershed areas with the highest amount of corn production and annual precipitation. Further, the methodology developed in this study has the potential for application in regions with higher dependency on surface water to determine agrochemical load influxes from upstream regions and evaluate other surface water contaminants in surface and/or groundwater.

Keywords: Herbicides; GIS modeling; Nitrate-N; Health Risk Assessment; MCLs;

Introduction

Water, an essential natural resource for agricultural production, is often threatened by the increasing use of pesticides to prevent crop damage (Vorosmarty et al., 2005). Majority of streams and rivers in the United States (U.S.) are ecologically impaired or threatened by anthropogenic stressors (Cardinale et al., 2012). These stressors, which include pesticide use, can lead to increased costs for water treatment (Velten et al., 2007), especially in states dependent on surface water. The use of pesticides can also result in the loss of biodiversity and ecosystem services (Vorosmarty et al., 2010). One major stream impairment in the Midwestern region of the U.S. is from water soluble, pre-emergent herbicides. Most of the pre-emergent herbicides used in the U.S. are within a 12-state area known as the “corn belt”, located in the Midwest (Gianessi & Puffer, 1991). Herbicides, such as atrazine, are persistent in agricultural environments and can undergo various fates, such as runoff into surface water, or slow processes of bio-decomposition (Shapir and Mandelbaum, 1997).

Atrazine (2-chloro-4-ethylamino-6-isopropyl-amino-s-triazine) is the second most commonly used herbicide in the U.S. as of 2012 (Atwood & Paisley-Jones, 2017) and one of the most commonly used pesticides in the world (Ackerman, 2007). For agricultural use alone, an average of 32.7 million kg (72 million lbs) of atrazine were applied annually from 2000-2010 in the U.S. (Spatz & Chie, 2016). Atrazine is also applied in the U.S. for non-agricultural uses, such as domestic-use weed killers, but is not ranked in the top 10 active ingredients (Atwood & Paisley-Jones, 2017). Typically, atrazine is applied to cornfields prior to emergence in order to control broadleaf and grassy weeds. For example, in 2014 atrazine was applied to 55% of planted acres of corn in the U.S., making it the most used pesticide for corn. Further, Nebraska planted 3.8 million hectares (9.3 million acres) of corn in 2014, comprising 10.3% of the corn production in the U.S. (NASS, 2015).

A special review conducted by the Environmental Protection Agency (EPA) in 1994 primarily focused on human health concerns and the potential effect of atrazine on non-target terrestrial and aquatic biota (USEPA, 1994). Atrazine was one of two herbicides detected most frequently in U.S. surface and ground water (Gilliom et al., 2006). Contamination of ground and surface and ground water also leads to contamination of our drinking water. For example, the US EPA National Survey of Pesticides in Drinking Water Wells found atrazine to be one of the most prevalent herbicide or pesticide in domestic water wells (Focazio et al., 2006; Ritter, 1990; Quackenboss et al., 2000). The persistent use of atrazine in the Midwest, along with the vast amount of applied fertilizers has led to the ubiquitous occurrence of these contaminants in waterways and created potential concerns for human exposure. When atrazine was identified as an endocrine disruptor, the concerns for human health arose (Forgacs et al., 2012). The International Agency for Research on Cancer (IARC) has found substantial evidence of carcinogenic effects of atrazine in experimental animal studies, but surmountable evidence has yet to be confirmed for its carcinogenicity on humans (International Agency for Research on Cancer, 1999). However, atrazine exposure has been shown to lower the serum and testicular testosterone and luteinizing hormone levels in rats (Stoker et al., 2000), feminize male frogs (Hayes et al., 2010), and, adversely impact human reproduction and birth outcomes (Rinsky et al., 2012; Munger et al., 1997; Ochoa-Acuna et al., 2009). Following these findings, the health implications from atrazine mixed with other contaminants, specifically nitrate-N (NO_3-N), in drinking water has continued to be investigated.

Occurrences of Non-Hodgkin Lymphoma (NHL) have been observed from exposure to both NO_3-N and atrazine in drinking water in the state of Nebraska (Rhoades et al., 2013). Specifically, Rhoades et al. (2013) analyzed drinking water quality data and considered exposure of NO_3-N and atrazine at lower doses. Exposure to NO_3-N were considered to be concentrations higher than the background level of 2 mg L^{-1}, while exposure to atrazine was considered at any detectable concentration, given that atrazine does not occur naturally in the environment. No association of a higher risk of NHL with exposure to either NO_3-N or atrazine alone were reported. However, dual exposure to both contaminants elevated NHL risk. The study later hypothesized that this cause of carcinogenesis was likely due to the *in vivo* formation of the nitrosamine *N*-nitrosoatrazine (NNAT) and its subsequent metabolism, which ensuing process has also been hypothesized (Ward et al., 2005; Cova et al., 1996; Krull et al., 1980). Nitrosamines have a variety of forms with many being known carcinogens (Pruessmann & Stewart, 1984). As humans ingest NO_3-N, the contaminant becomes reduced to nitrite (NO_2-N) in the stomach, allowing secondary amines to be nitrosated due to the acidity found in human stomachs (Mirvish, 1975; Wolfe et al., 1976; Loeppky, 1994; Brambilla et al., 2009). Atrazine is a secondary amine and, when also present, allows nitrosates to form NNAT, which has been linked to a higher risk of chromosomal abnormalities in lymphocytes *in vitro* at doses as low as 0.0001 $\mu g\ mL^{-1}$ (Meisner et al., 1993). Additionally, atrazine and NO_3-N have also been found to impact prenatal health. Stayner et al. (2017) analyzed the risk of preterm delivery (PTD, < 37 weeks), very preterm delivery (VPTD, < 32 weeks), low birth weight (LBW, < 2.5 kg among infants born at term), and very low birth weight (VLBW, < 1.5 kg) in four Midwestern states following exposure to atrazine and NO_3-N, both separately and together. Data was obtained from 134,258 births in 2008 from 46 rural counties in the Midwest with public water systems that were a part of the U.S. EPA atrazine monitoring program (AMP). The data used for this study was obtained from four states (Ohio, Indiana, Iowa, and Missouri) and was analyzed to estimate rates of PTD, VPTD, LBW, and VLBW and compare rates with the local monthly concentrations of atrazine and NO_3-N in drinking water. Exposure to atrazine was found to impact prenatal health between 4 to 6 months prior to birth for PTD, and for VPTD between 0 to 3 months. For NO_3-N exposure, there was also evidence that suggested a linear exposure-response relationship for the risk of VPTD, VLBW and exposure to NO_3-N in drinking water. However, VPTD was most significant ($p=0.001$) during periods which atrazine and NO_3-N were both contaminants in the water 0 to 3 months prior to birth.

The magnitude of past research regarding ecotoxicity of these pre-emergent herbicides are as primary threats to non-target aquatic species. Reported studies have shown interactions with atrazine impact: aquatic microorganisms by radically altering community structure (Graymore et al., 2001), fish by endocrine disruption (Fan et al., 2007), and amphibians by inducing hermaphroditism in exposed males (Hayes et al., 2002). The EPA's Office of Pesticides Programs (OPP) set aquatic life benchmarks for freshwater species to standards developed during pesticide registration. The OPP's Aquatic Life Benchmark freshwater acute concentrations for atrazine are 2,650 μg L^{-1}, 360 μg L^{-1}, <1 μg L^{-1}, and 4.6 μg L^{-1} for fish, invertebrates, nonvascular plants, and vascular plants, respectively. The OPP's Aquatic Life Benchmark freshwater chronic concentrations for atrazine are 5 μg L^{-1} for fish and 60 μg L^{-1} for invertebrates.

Based on the potential health and ecological implications of atrazine and NO_3-N being present in surface water, further investigation is needed to identify "hot" times and "hot" spots in in the Midwest. The novelty of this study is that it is one of the first to implement a dual risk factor methodology concerning two different types of contaminants; therefore, the objective of this study was to provide a case study for completing an environmental risk analysis for the possible exposure of atrazine to ecosystems and humans through interaction with surface waters. The objective was met with two approaches: (1) Identify watersheds across Nebraska that were at risk for exceeding nitrate-N and atrazine maximum contaminant limits (MCLs) in surface water; and (2) Determine the specific times in the year where risks were greatest.

Materials and Methods

Data Acquisition

Surface water samples were collected by the Nebraska Department of Environmental Quality (NDEQ) at 68 selected sites in Nebraska throughout the year for 12 years (2003-2014). Water quality concentrations and locations (latitude and longitude) were recorded by station number in the NDEQ's STORET database. These samples followed the US EPA guidelines for procurement and analysis of surface-water samples. Atrazine from surface water grab samples was analyzed using quantitative immunoassay (EPA SW-846 Test Method 4670). The product utilized in this method by the NDEQ was the Abraxis® Atrazine, ELISA Kit using the Microtiter Plate. This method involved mixing a known volume of the grab sample with an enzyme-atrazine conjugate reagent in a test tube that contained an anti-atrazine antibody immobilized on the surface. The conjugate competed with the available atrazine to bind to the anti-atrazine immobilized antibody. The test tube was then incubated for 30 minutes. The unbound conjugate and sample analyte were then washed from the test tube with organic-free reagent water. A signal generating substrate was then added to the solution and incubated. In some cases, a magnetic field was required to retain the magnetic particle coated with antibody during the wash. For the immunoassay, a stop solution was added to the test tube to halt the signal generating activity caused by the enzyme reagent. The absorbance of the solution was then measured at a specific wavelength and results were interpreted using analytical standards (USEPA, 2007).

The NO_3-N was measured using the US EPA Method 353.2 by the NDEQ. This method determines NO_3-N and NO_2-N concentrations by automated colorimetry. The filtered water sample passes through a granulated copper-cadmium column to reduce NO_3-N to NO_2-N. The reduced NO_3-N and the original NO_2-N present is calculated by analyzing the highly colored azo dye produced when the NO_2-N diazotizes with the sulfanilamide when coupled with the N-(1-naphthyl)-ethylenediamine dihydrochloride. All NO_3-N samples collected were analyzed at the Nebraska state laboratory in Lincoln, Nebraska.

The 68 available monitoring stations used in this study are shown in Figure 2.1. Sites were chosen based on their location, and available data for both atrazine and NO_3-N.

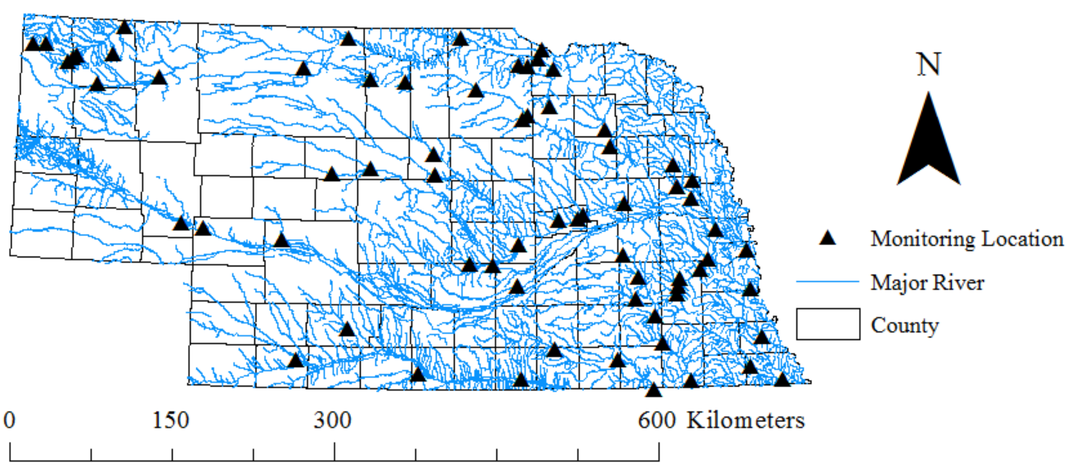

Figure 2.1: *Surface water monitoring locations for atrazine and NO_3-N by the Nebraska Department of Environmental Quality.*

Single Risk Analysis

A risk factor was determined for each monitoring location based on the US EPA MCL of atrazine and NO_3-N from the available surface water quality data from the NDEQ. The risk analysis followed methods developed by Di Guardo and Finizio (2017) for glyphosate, another very commonly used pesticide, where risk factors for each surface water station were used to analyze the safety of watersheds in the Lombardy region of Italy. In our study, we adapted the methodology to determine atrazine and NO_3-N exposure risks within Nebraska. While the current MCLs for atrazine and NO_3-N in the U.S. are 3 μg L^{-1} and 10 mg L^{-1}, respectively, NO_3-N exposure limits identified in Rhoades et al., 2013 for increased risk of NHL (2 mg L^{-1} for NO_3-N) were also used in this study, while the atrazine exposure value was kept at the EPA's MCL for drinking water. The data was analyzed by calculating an annual risk factor for each selected monitoring location and was spatially correlated using GIS tools in ArcMap.

The equation to determine the risk factor (RF) for atrazine and NO_3-N was:

$$\mathrm{RF}_{\mathrm{MCL}}^{95\mathrm{th}\%} = \frac{95\mathrm{perc}(\mathrm{MEC_i})}{\mathrm{MCL}},$$

where MEC_i was the measured environmental concentration at time *i*, 95perc(MEC_i) was the 95th percentile value of MEC, MCL was the maximum contaminant level, and $\mathrm{RF}_{\mathrm{MCL}}^{95\mathrm{th}\%}$ was the risk factor based on the 95th percentile of the MEC and MCL. The RF is designed to be unit-less, so the unit represented for the MCL should reflect the same for the unit utilized in determining the 95th percentile for each pollutant (μg L^{-1}, mg L^{-1} for atrazine and nitrate, respectively). The measured environmental concentration (MEC) was the individual concentrations that were obtained from NDEQ, while the 95th percentile the dataset was used for each watershed to ensure that the maximum concentrations of atrazine and NO_3-N were weighted more heavily than the lower concentrations to account for seasonal herbicide applications. The RF was assessed for each calendar year to determine possible trends in risk within in each monitoring location. This same methodology was used for NO_3-N concentrations. The individual RF's for both atrazine and NO_3-N were divided into four classes of risk for each year and analyzed: $RF_{MCL}^{95th\%}$ less than 0.8 was considered safe; 0.8 to 1.0 was considered at low risk; 1.0 to 2.0 was considered at risk and greater than 2.0 was considered at high risk. The 95th percentile MEC values and their corresponding risk factors are shown below (Table 2.1). One atrazine risk factor and two NO_3-N risk factors (10 and 2 mg L^{-1}) were used to determine dual exposure risks.

Table 2.1: *Atrazine and NO_3-N 95th % concentrations and associated risk factors*

Risk Level (RI)	Risk Factor Range	NO_3-N (MCL 10 mg L^{-1})	NO_3-N (MCL 2 mg L^{-1})	Atrazine (MCL 3 μg L^{-1})
		95th % Concentration (mg L^{-1})	95th % Concentration (mg L^{-1})	95th % Concentration (μg L^{-1})
Considered Safe (0)	0.0-0.8	0-8	0.0-1.6	0.0-2.4
Low Risk (1)	0.8-1.0	8-10	1.6-2.0	2.4-3.0
Risk (2)	1.0- 2.0	10-20	2.0-4.0	3.0-6.0
High Risk (3)	> 2.0	> 20	> 4.0	> 6.0

Dual Risk Analysis

Risk factors and interpolation methods provided by ArcMap (ESRI, 2014) were then used to assess trends in atrazine, NO_3-N, and combined atrazine and NO_3-N exposure risks spatially for the monitoring locations from 2003-2014. The combined, dual risk factor (DRF) used in this study was developed to assess the risk of dual exposure of both NO_3-N and atrazine in surface water. The DRF was calculated by:

$$\mathrm{DRF} = \mathrm{RI}(x_1) + \mathrm{RI}(x_2)$$

where RI(x) is the risk integer of contaminant (x) and DRF is the dual risk factor. Specifically, in this new methodology for determining risk from dual exposure, the DRF ranged from 0 (considered safe for both atrazine and NO_3-N exposure) to 6 (at high risk for both atrazine and NO_3-N exposure). A general term was applied to these different integer values for dual risk factor: 0 = Very Low Risk; 1 = Low Risk; 2 = Medium-Low Risk; 3 = Medium Risk; 4 = Medium-High Risk; 5 = High Risk; and 6 = Very High Risk.

Interpolated Maps

The interpolation tool in ArcMap used for this study was the inverse distance weighted method, or IDW (ESRI, 2014). The usefulness of the IDW method was for estimating unknown values between known values, while considering areas surrounding these known points to be the most heavily influenced by their values. This influence of each value was lessened as distance was increased between sampling points. In the context of this study, when examining risk factors for each monitoring station, if a region had higher risk factors for atrazine or NO_3-N, then IDW would predict the surrounding area between the monitoring locations to have similar risk factors. If locations were farther away from known points, their risk factor was affected less. Risk factor values were mathematically estimated using the IDW method in regions where data collection was minimal by using the nearby known data points to create interpolated maps.

The interpolated maps were created for atrazine, NO_3-N, and dual risk factors using both NO_3-N MCL values of 10 and 2 mg L^{-1}. The purpose of exploring both the 10 and 2 mg L^{-1} contaminant values for NO_3-N was to show the difference in reducing the modeled

MCL value when considering two pollutants. Reducing the modeled MCL for the interpolated DRF maps would be representative of considering two pollutants simultaneously, which could have effects when combined that are not examined when observed singularly. Interpolated maps have their benefits as well as drawbacks. The maps have the potential to be useful tools to determine trends and to maximize efforts of containing and mitigating pollutants in the environment. However, the maps also project mere estimations. In this study, the main goal of creating interpolated maps was to draw attention to the possible chronic detrimental risk from being exposed to atrazine and NO_3-N from surface waters across Nebraska.

Results

Atrazine and Nitrate

Atrazine and NO_3-N data from the 68 monitoring locations were used to complete risk assessments throughout time and space. These risk assessments provided consistency between comparisons on annual individual agrochemical exposures. Surface water data was analyzed each year for average and maximum atrazine concentrations, number of viable samples, and first and last sampling events each year (Table 2.2).

Table 2.2: *Overview of collected samples that comprised the atrazine surface water dataset.*

Year	Average Atrazine Concentration (μg L^{-1})	Max Atrazine Concentration (μg L^{-1})	Standard Deviation (μg L^{-1})	# of Viable Samples	First Day of Sampling	Last Day of Sampling
2003	1.88	175.74	7.73	3139	1/6/2003	12/30/2003
2004	1.26	105.04	5.18	2096	1/12/2004	12/14/2004
2005	1.22	80.80	5.60	1466	1/10/2005	10/13/2005
2006	0.54	85.85	3.16	2172	1/3/2006	11/14/2006
2007	2.45	167.50	9.38	1929	4/2/2007	12/13/2007
2008	0.72	76.00	2.81	1409	1/3/2008	12/2/2008
2009	1.03	87.15	3.67	1297	4/6/2009	9/29/2009
2010	1.71	60.94	4.82	1266	4/5/2010	9/30/2010
2011	0.71	29.82	2.06	500	4/4/2011	9/29/2011
2012	1.56	100.17	6.37	1342	4/2/2012	9/27/2012
2013	1.48	123.41	6.48	1416	5/1/2013	10/1/2013
2014	0.95	81.48	4.26	1090	5/1/2014	9/30/2014
Average	**1.29**	**97.83**		**1594**		

From 2003-2014, a decreasing trend in the number of viable samples were collected, as well as a reduction in sampling periods (Table 2.2). Majority of atrazine concentration spikes occurred between April-July. However, beginning in 2013 sampling for atrazine did not begin until the first week of May, potentially missing the peak atrazine concentrations. Sample frequency at each site was approximately four weeks, which had the potential to allow first flush samples to be lost prior to the following sampling event.

Surface water NO_3-N concentrations for the 68 selected sites were statistically analyzed from 2003-2014 (Table 2.3). Similar to atrazine, the analysis included overall annual average and maximum surface water concentrations, number of viable samples, and the first and last day of collected samples for each year. However, NO_3-N concentrations were sampled at a higher frequency in comparison to atrazine. This common trend resulted in more consistent and complete datasets of NO_3-N compared to atrazine, which relied on inconsistent sampling periods from year to year, and sampling intervals throughout the year. NO_3-N is often contributed to streams through groundwater, resulting in less dependence on timing following a storm event to acquire a meaningful sample. To further assess the annual risk of atrazine and NO_3-N exposure, yearly risk assessments were completed using the IDW method from 2003-2014 (Figures 2.2a and 2.2b), where increased darkness correlated to increased regional risk.

Table 2.3: *Overview of collected samples that comprised the NO_3-N surface water dataset.*

Year	Average Nitrate Concentration (mg L^{-1})	Max Nitrate Concentration (mg L^{-1})	Standard Deviation	# of Viable Samples	First Day of Sampling	Last Day of Sampling
2003	2.11	18.20	2.49	1024	1/6/2003	12/3/2003
2004	2.03	24.67	2.70	1282	1/12/2004	12/14/2004
2005	2.10	25.45	2.80	1287	1/10/2005	12/8/2005
2006	1.85	57.18	2.88	1234	1/9/2006	12/6/2006
2007	1.96	47.50	2.84	1322	1/8/2007	12/6/2007
2008	1.97	24.50	2.46	1049	1/7/2008	12/4/2008
2009	2.29	29.27	2.68	919	1/6/2009	12/18/2009
2010	2.23	46.28	2.70	2102	1/4/2010	12/8/2010
2011	2.24	33.60	3.05	1906	1/3/2011	12/14/2011
2012	2.21	30.19	2.70	1849	1/3/2012	12/6/2012
2013	2.09	47.50	2.86	1873	1/7/2013	12/17/2013
2014	1.94	28.70	2.71	1006	1/6/2014	12/9/2014
Average	**2.09**	**34.42**		**1404**		

The atrazine risk factor maps remained consistent throughout the assessed years with the exception of a four year window from 2008 to 2011, where the risk was strongly reduced. The 2010 and 2011 atrazine risk factor maps were not fully covered interpolated maps due to a severe lack of available data in the western part of Nebraska.

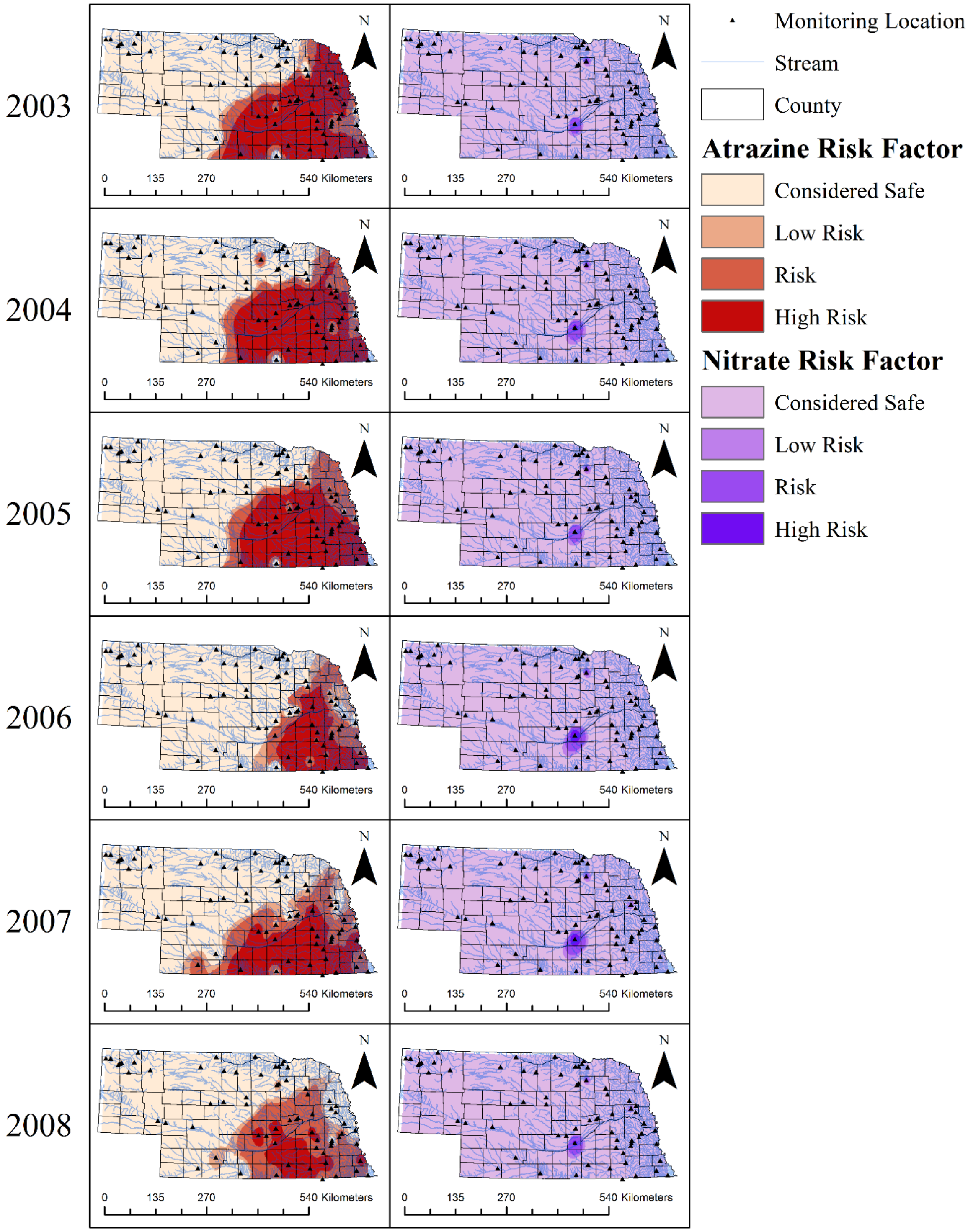

Figure 2.2a*: Interpolated maps of atrazine (left) and NO_3-N (right) risk factors for years 2003-2008 with risk factor maximum contaminant levels set for drinking water standards (3 µg L^{-1} and 10 mg L^{-1} for atrazine and NO_3-N, respectively).*

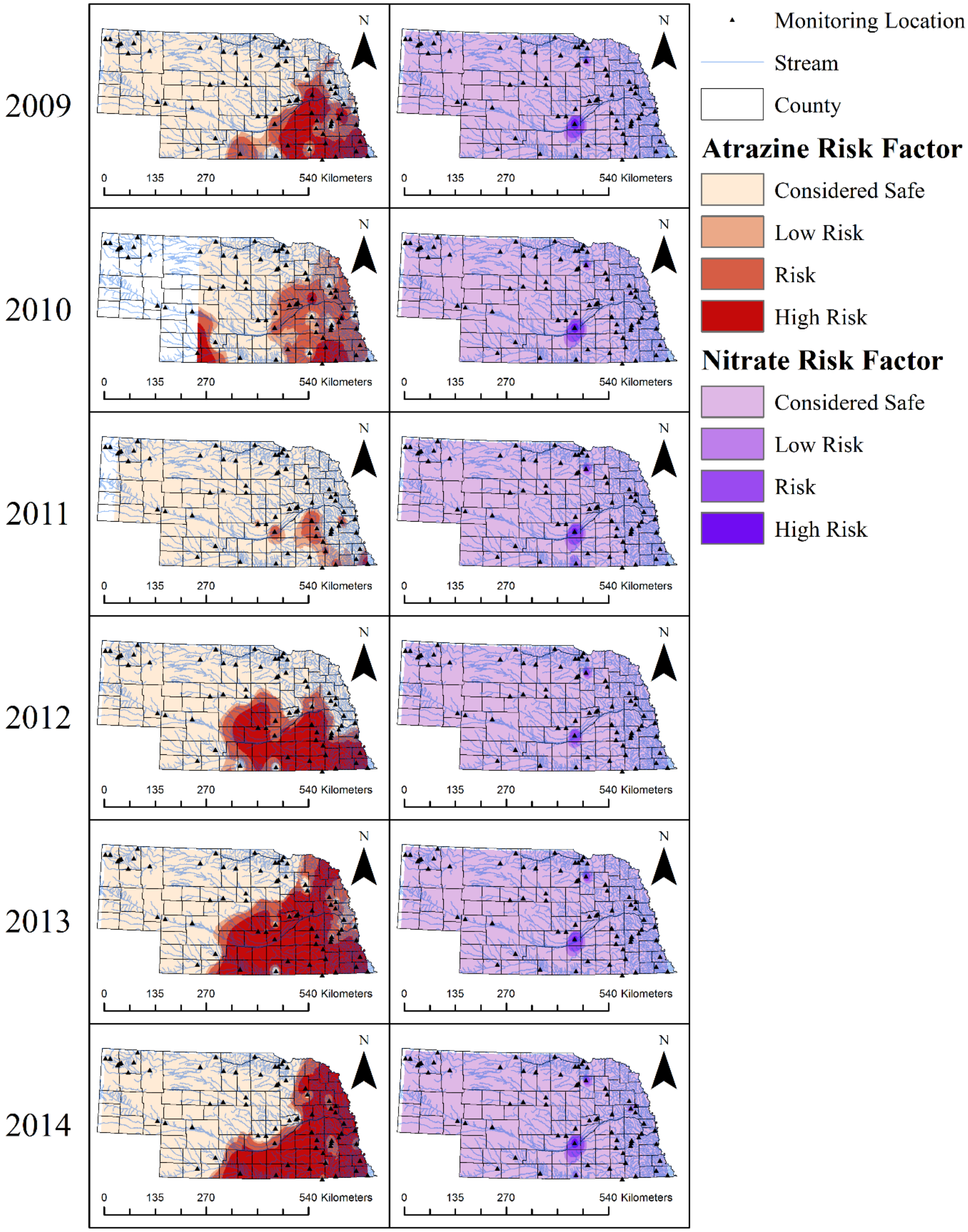

Figure 2.2b: *Interpolated maps of atrazine (left) and NO_3-N (right) risk factors for years 2009-2014 with risk factor maximum contaminant levels were set for drinking water standards (3 μg L^{-1} and 10 mg L^{-1} for atrazine and NO_3-N, respectively).*

The NO_3-N risk factor maps remained consistent, identifying only one major "hot spot" in Nebraska where a point source is located. This point source is labelled "Outfall 001" from the Swift Beef company, which lost a civil suit against the U.S. and the State of Nebraska in 2012 for violating the Clean Water Act (U.S. Department of Justice, 2011). As a result, the company had to pay $1.3 million for restitution.

Dual Exposure

Atrazine and NO_3-N are both agrochemicals considered vital for crop production and crop insurance. Therefore, these agrochemicals were expected to be spatially similar within Nebraska. Timing of chemical applications was crucial for determining the dual risk of exposure and potential "hot" spots and times of both atrazine and NO_3-N. Although the maps were determined using data from the entire span of the year, atrazine and NO_3-N were applied at similar times of the year and were often present in surface water around the same time as well. The DRF (10) and DRF (2) were associated with using the 3 $\mu g\ L^{-1}$ for atrazine and 10 $mg\ L^{-1}$ and 2 $mg\ L^{-1}$ for NO_3-N, respectively (Figures 2.3a and 2.3b).

The dual risk factor evaluated the impact of alterations to NO_3-N concentration on the overall dual exposure risk. For example, examining the DRF at 10 $mg\ L^{-1}$ NO_3-N in comparison to 2 $mg\ L^{-1}$ NO_3-N, resulted in substantially fewer regions at risk. Further, hot spots identified in the dual exposure assessment exhibited a pattern in terms of spatial distribution across the state of Nebraska. The locations that showed the highest risk of atrazine exposure were located in the southeastern part of the state.

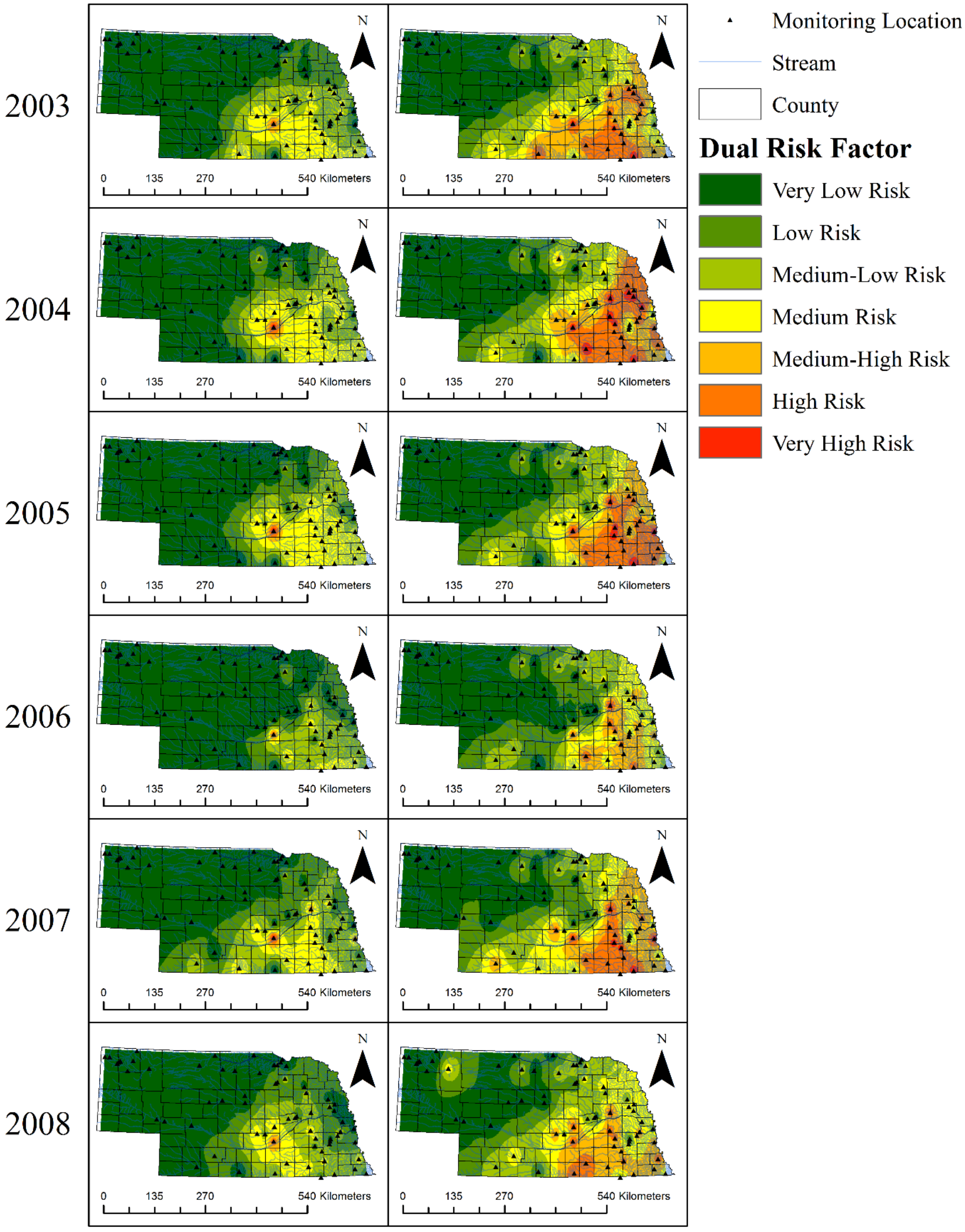

Figure 2.3a: *Dual risk factor interpolated maps for atrazine and NO_3-N for 2003-2008 with NO_3-N concentrations of 10 mg L^{-1} (left) and 2 mg L^{-1} (right).*

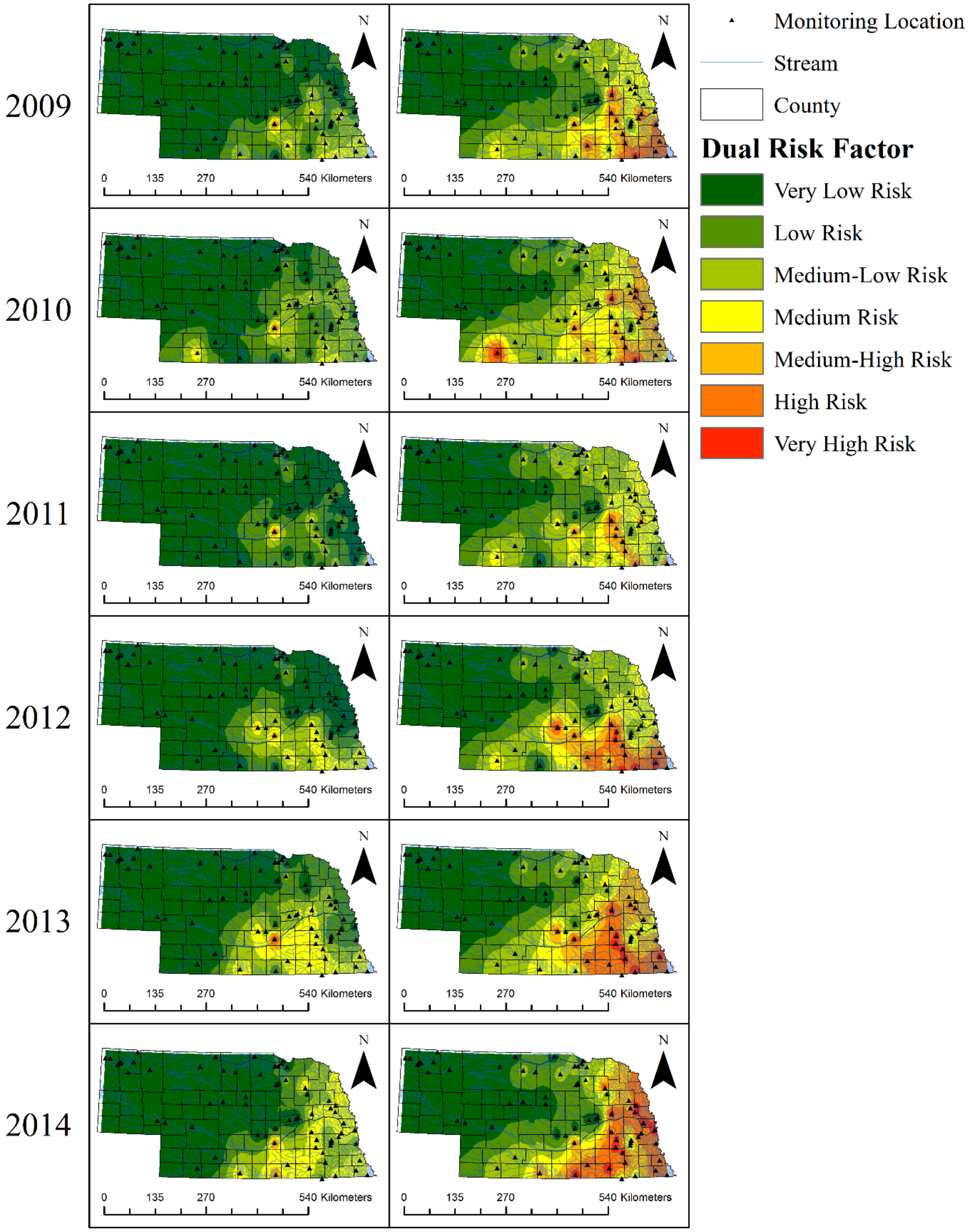

Figure 2.3b: *Dual risk factor interpolated maps for atrazine and NO_3-N for 2009-2014 with NO_3-N concentrations of 10 mg L^{-1} (left) and 2 mg L^{-1} (right).*

Discussion

Consistent trends were observed for potential risks of exposure throughout the year and in specific regions of Nebraska. Factors that likely impacted increased risks of dual exposures included precipitation following application and crop production. Other researchers have reported similar impacts on agrochemical exposure from precipitation caused overland flow (Hyer et al., 2001; Huber, 1993). Therefore, precipitation data was interpolated from various weather stations for each year, specifically during the growing season. Average annual atrazine concentrations were calculated for each monitoring location to help identify trends between the precipitation data and the prevalence of exposure risk to atrazine. Corn production land use was also examined using the 2014 US National Agricultural Statistics Service (NASS, 2015) data to localize trends in the risk factor interpolated maps.

Surface Water Impact

A 2015 study conducted by USGS, reported Nebraska used approximately 1,040 million liters (275 million gallons) of water each day for its public water supply. While the majority of this water is pumped groundwater from the Ogallala aquifer, 20.8% of the water withdrawals come from surface water (Dieter et al., 2018), specifically in the eastern region of Nebraska. Water drawn from surface water sources in regions like eastern Nebraska, would have a higher probability of being contaminated with atrazine or any of its byproducts or degradants and would require additional costs for water treatment (Velten et al., 2007).

Rural communities often have the greatest challenges dealing with these surface water impairments to meet compliance with regulatory water quality standards due to limitations in technical expertise and financial resources. A new study by Allaire et al., (2018) found that the compliance gap was substantial when comparing low-income rural communities with urban communities. Therefore, rural Midwestern municipalities downstream from dominantly corn producing regions, likely are challenged when treating emerging contaminants such as atrazine due these limited resources.

Spring Flush

Precipitation data was assessed to estimate “spring flush” using the accumulated precipitation from April through July. Spring flush, a phenomenon where the early rains of the growing season often follow application of fertilizers and pesticides, has been found to be the most likely period to observe the transport of agrochemicals to surface water bodies especially in the Midwest for atrazine (Thurman et al., 1991). NO_3-N concentration has also been shown to increase following storm events, usually lagging in time several hours compared to other agrichemical constituents (Hyer et al., 2001). Battaglin et al., (2003) found that in Midwestern streams 90% or more of the total herbicide load was contributed from runoff and less than 10% from groundwater discharge. The spring flush phenomenon for atrazine repeatedly has been observed for over two decades due to its water solubility and early spring application period (Rinsky et al., 2012) and resulting in spikes in concentration in surface water during the early summer months (Stayner et al., 2017). Similarly, Louchart et al. (2001) found that the loss of herbicides from the field and watershed scale were due to intense storm events, with highest herbicide concentrations in runoff occurring during the first rainfall event. In these studies, herbicide concentrations gradually decreased following precipitation events, but remained above 1 ppb for several months. To analyze the seasonal fluctuations in our study, all surface water atrazine concentrations throughout Nebraska from 2003-2014 were compiled and plotted over the Julian days of the year. Spring flush occurred between April and July each year, during which time higher atrazine concentrations were regularly observed (Figure 2.4). Furthermore, the highest measured atrazine concentrations were over 50 times the EPA's MCL concentration for drinking water.

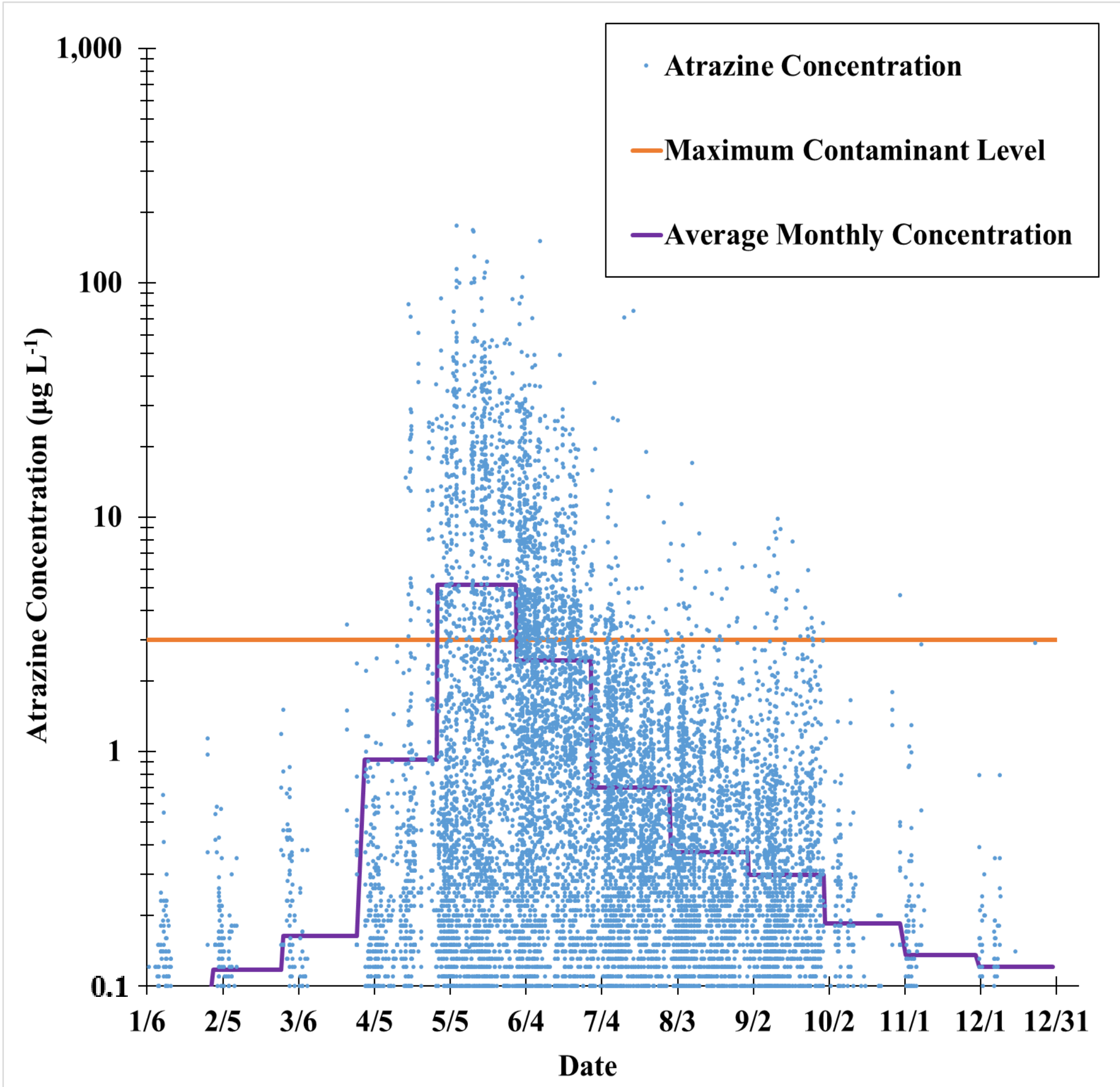

Figure 2.4: *Surface water concentrations of atrazine in Nebraska from 2003-2014.*

Further trends of exposure risks were observed to be associated with accumulated precipitation between April and July of each year (Figures 2.5a and 2.5b). Precipitation varies across Nebraska, with the western part receiving between 400 mm to 450 mm (15.8" to 17.7") annual precipitation and the eastern portion of the state receiving as much as 800 mm to 850 mm (31.5" to 33.5") annual precipitation (Arguez et al., 2010). The location and timing of precipitation was observed to greatly impact the risk factor associated with atrazine exposure. The western part of the state is a lot dryer, due to less annual precipitation and sandier soils, which also means that there are fewer row crops and corn production resulting in less agrochemical application, while higher production of corn and rainfall was observed in the eastern portion of Nebraska. Similarly, Troiano et al. (1993) and Steenhuis et al. (1994) found pesticide mobility in soil was directly related to increased water transport in soils.

Therefore, annual precipitation maps were compared with the atrazine risk factor maps in Nebraska for this study. Years with less precipitation had a smaller area of risk for atrazine, while NO_3-N was not influenced from the change in spring flush magnitude likely due to its overall ubiquitous nature throughout the state and is primarily transported through groundwater rather than surface runoff. Spahr et al (2010) found that across the US, 40% of the study sites had nitrate load contributions that were higher than 50% from base flow. Especially in the Northern Plains, such as Nebraska, most base flow contribution ratios for nitrate load were larger than 50%. The Dismal River in Nebraska is a well-documented study site that had about 98% of the nitrate load contributed from base flow.

Additional trends that were visible in the interpolated precipitation and average atrazine concentrations maps (Figures 2.5a and 2.5b) included: (1) wet years during April-July in the eastern part of Nebraska lead to dilution of atrazine in surface waters and a decrease in average atrazine concentrations; and (2) severe droughts in the eastern part of the state, such as the drought in 2012, lead to an increase in surface water atrazine concentrations the following year, likely due to less organic material to provide absorption sites. Laird et al. (1994) found that atrazine primarily attaches to silicate clay materials but has increased retention ability with the presence of more soil organic matter.

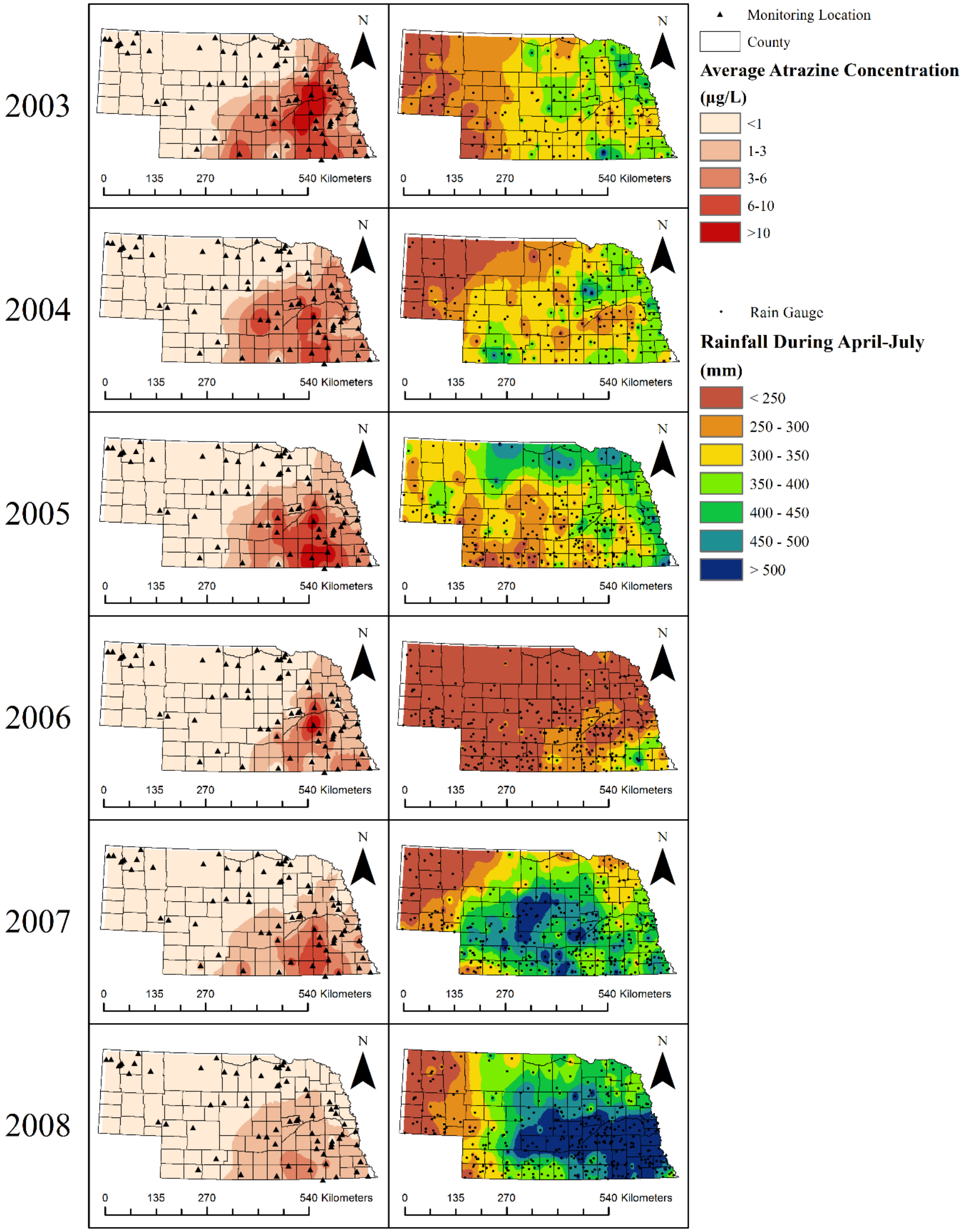

Figure 2.5a: *Average atrazine concentrations (left) and average accumulated precipitation (right) in Nebraska from April to July for the years 2003-2008.*

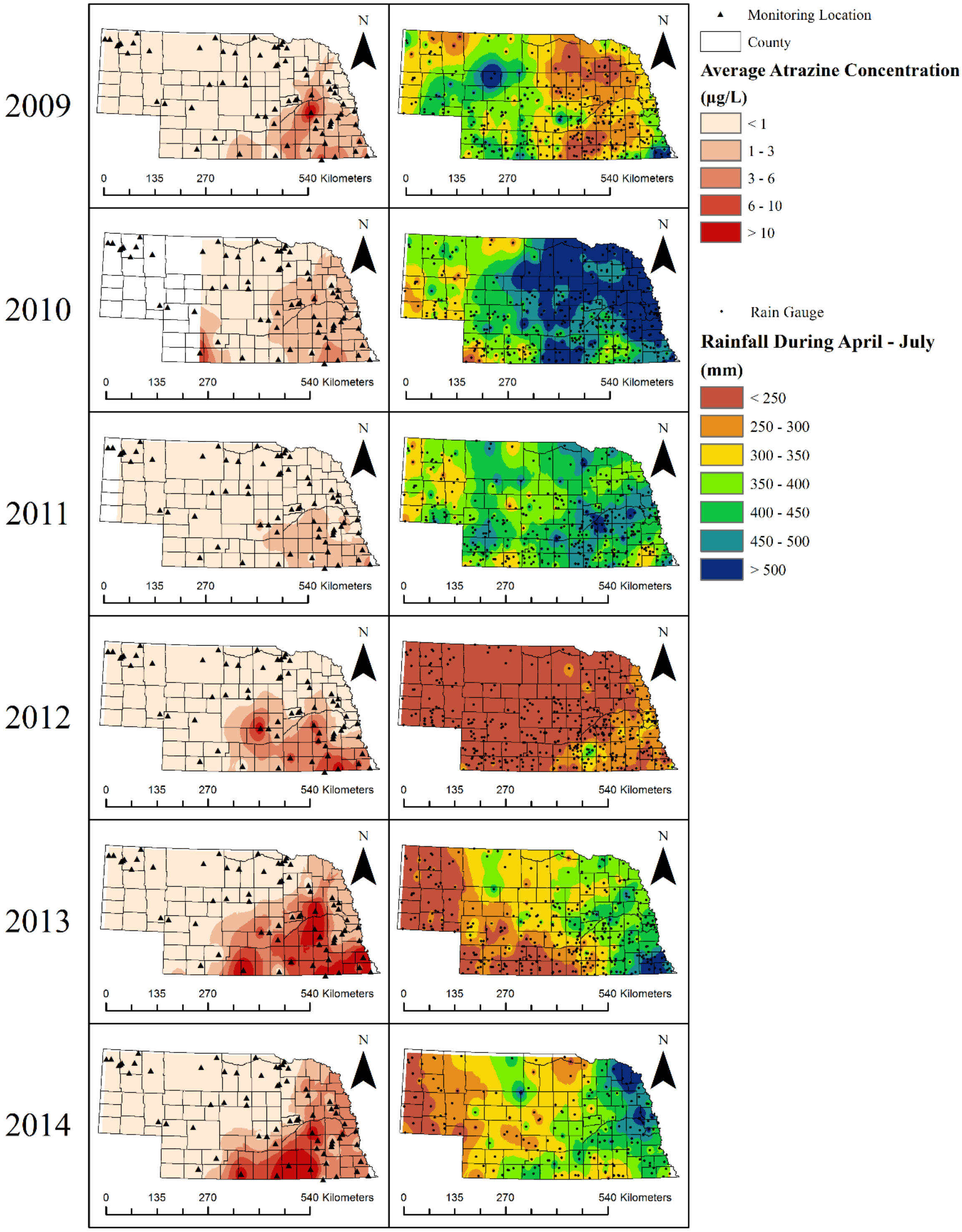

Figure 2.5b: *Average atrazine concentrations (left) and average accumulated precipitation (right) in Nebraska from April to July for the years 2009-2014.*

Acute Toxicity

High concentrations of atrazine, as observed in this study during the spring flush phenomenon, have the potential to adversely impact non-target aquatic biota, which are usually more affected by agrochemical pulses from runoff events (Ferenczi et al., 2002). The highest recorded atrazine concentrations were 175 µg L^{-1}, which is about 1/15 (6.6%) of the acute fish toxicity level (2650 µg L^{-1}) and approximately 1/2 (48%) of the acute invertebrate toxicity level (360 µg L^{-1}). However, average yearly atrazine concentrations for all of the selected monitoring locations across the state exceeded acute toxicity levels for nonvascular plants (1 µg L^{-1}) 8 out of 12 observed years. The yearly maximum concentration for vascular plants continually exceeded the acute toxicity level of 4.6 µg L^{-1} from April to October for all evaluated years (Figure 2.4; Table 2.4).

Table 2.4: *Monthly average and maximum atrazine concentrations across Nebraska from 2003-2014 by Nebraska Department of Environmental Quality of the 68 monitoring locations.*

Month	Average Atrazine Concentration (µg L^{-1})	Maximum Atrazine Concentration (µg L^{-1})	Standard Deviation Concentration (µg L^{-1})
January	0.07	1.14	0.10
February	0.12	1.19	0.46
March	0.16	3.49	0.55
April	0.92	80.8	6.09
May	5.16	175.74	18.22
June	2.45	150.49	6.22
July	0.70	76.00	2.18
August	0.37	17.00	0.72
September	0.30	9.84	0.72
October	0.18	4.66	0.37
November	0.13	2.86	0.23
December	0.10	2.90	0.22

Chronic Toxicity

Rhoades et al. (2013) suggested that exposure to atrazine and NO_3-N simultaneously in drinking water was most likely not an acute toxicological effect, but a chronic effect. Lim et al., (2009) found that a chronic exposure of atrazine at low concentrations induced abdominal obesity and insulin resistance in rats. Similarly, the chronic exposure to sensitive invertebrates has the potential to impact the overall aquatic ecosystems within hot spot and downstream regions where atrazine concentrations remain high. Ralston-Hooper et al. (2009) reported LC_{50} for atrazine decreased significantly following a change from acute to chronic exposure for benthic amphipods *Diporeia* spp. The average recorded atrazine concentration in this study was 1.29 µg L^{-1} (Table 2.1), which is about ¼ of the atrazine chronic fish toxicity level (5 µg L^{-1}). However, between 2003-2014, there was only one year (2011) where maximum atrazine concentrations did not reach the 60 µg L^{-1} chronic toxicity level for invertebrates. The highest recorded atrazine concentration in this study was almost 3X (2.9) the chronic toxicity level for invertebrates.

Crop Cover

The location and intensity of row crop land use was also examined given atrazine is a primary herbicide used for corn production (Figure 2.6). A strong correlation between the normal risk factor spatial distribution of atrazine and corn production was observed. The monitoring locations in the western part of Nebraska never reached a level of risk from exposure because atrazine was not as extensively applied in this region. The southeastern part of the state had the most consistent levels of risk for atrazine exposure due to the prevalence of corn production in the area. When both atrazine and NO_3-N were considered in the dual risk factor (10) and dual risk factor (2), the consistent "hot spots" still remained in the areas of Nebraska where corn production was most prevalent.

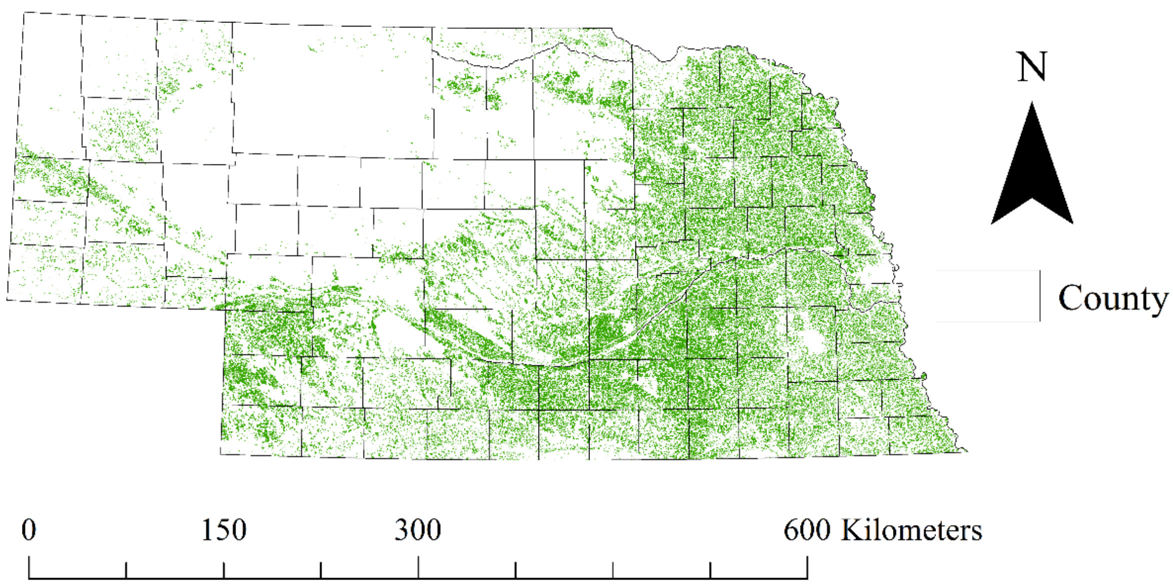

Figure 2.6: *2014 National Agricultural Statistics Service map of corn production throughout Nebraska.*

Conclusion

In this study, the dual risk exposure to atrazine and NO_3-N in surface water was assessed. Atrazine was found to be susceptible to the spring flush effect. During years with dry springs, the risk in exposure to atrazine and NO_3-N were reduced. However, the reduction in the dual risk factors were due to the decrease in risk of atrazine exposure. The risk factor associated with NO_3-N exposure remained consistent in its spatial distribution throughout this study. Further, the atrazine risk was observed in regions of Nebraska with high corn production, which resulted in these regions subsequently resulting in higher risks for dual exposure to atrazine and NO_3-N.

The methodology presented has the potential to increase assessment and awareness of dual exposure risks from multiple contaminants and alter current risk assessment methods. Further, as the increasing demand for real-time data and analysis continue, this methodology has the potential to be utilized with real-time data to re-create interpolated risk maps throughout the year with improved precision. Lastly, the presented methodology could be applied for assessing load removal requirements in water treatment plants in surface water dependent regions and could be expanded to groundwater risk assessments.

Acknowledgements

Collaborators that made this project possible include the Nebraska Department of Environmental Quality. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the Nebraska Department of Environmental Quality. Special thanks to Dr. Kent Eskridge for statistical consultation on this project.

CHAPTER 3: *ESCHERICHIA COLI* HOT SPOTS AND HOT TIMES IN A RESERVOIR SYSTEM IMPACTED BY CATTLE GRAZING AND MIGRATORY WATERFOWL

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Abstract

Recent pathogenic *Escherichia coli* contamination of vegetables that originated from irrigation has increased awareness of identifying sources of *E. coli* entering these systems. However, limited methods for accurately predicting *E. coli* occurrence and sources in waterways continue to limit the identification of appropriate and effective prevention and treatment practices. Therefore, the primary objectives of this study were to: (1) Determine the load of *E. coli* during storm events in a hydrologic controlled stream situated adjacent to a livestock grazing operation that is located in the Central Flyway for avian migration in the Midwest and (2) Identify trends between *E. coli* concentrations, grazing rotations, and avian migration patterns. The study sampled five rainfall events (three summer events and two fall events) to measure the *E. coli* concentration throughout the storm events. A complex combination of bovine density and waterfowl migration patterns were found to significantly impact *E. coli* concentrations in stream water. Bovine density had a significant impact during the summer season ($p<0.0001$), while waterfowl density had a significant impact on *E. coli* concentrations during the fall ($p=0.0422$). The downstream reservoir had exceedance probabilities above the EPA freshwater criteria >85% of the growing season following rainfall events. Based on these findings, implementation of best management practices for reducing *E. coli* concentrations during the growing season and irrigation water testing prior to application are recommended.

Keywords: *E. coli*; Nitrate-N; Phosphate-P; Surface Water Monitoring;

Introduction

Surface water is often threatened by pathogens such as *Escherichia coli* (Wilkinson et al., 2011; Pendergrass et al., 2015; Gagliardi and Karns, 2000). *E. coli* is a bacterium found in the intestines of both people and warm-blooded animals. Therefore, it is used as an indicator for fecal contamination, and thereby the likelihood of pathogens to be present in water bodies. While most strains of *E. coli* are harmless commensals, the *E. coli* O157:H7 strain produces Shiga toxins and is own of the most harmful strains of *E. coli*. The Shiga toxin-producing *E. coli* O157:H7 strain, can result in intestinal infections, dehydration, kidney failure, and death. In agricultural settings, surface water bodies often become contaminated with pathogens from manure application, and grazing livestock in adjacent fields (Gagliardi and Karns, 2000; Wilkinson et al., 2011; Derlet et al., 2012), and avian presence in waterways (Pendergrass et al., 2015). These stressors have led to increased water treatment costs (Velten et al., 2007, Rompre et al., 2002), especially for municipalities dependent on surface water.

Approximately $600 million were spent annually in the U.S. on medical expenses related to *E. coli* O157:H7 infections, not including the other cases of exposure to non-O157:H7 strains of pathogenic *E. coli* (Scharff, 2012). In 2010 alone, within the U.S. there were 63,153 cases, 2,138 hospital admittances, and 20 deaths from *E. coli* O157:H7 illness. Children and the elderly are especially vulnerable to *E. coli* O157:H7 exposure from the complication of hemolytic uremic syndrome. This disease occurs in 2 to 7 percent of infections and is extremely hazardous to humans due to kidney failure. Hemolytic uremic syndrome (HUS) is the main cause of acute kidney failure in children in the U.S. and the main culprit is exposure to *E. coli* O157:H7 (Siegler, 1995). Children and females are the most susceptible to developing HUS from *E. coli* O157:H7 infection. Mortality rates for HUS, dependent on age, average approximately 4.6% (Gould et al., 2009).

E. coli continues to contaminate reservoirs in both agricultural and urban aquatic ecosystems, which results in further food security and health implications (Soller et al., 2010; Efting et al., 2011). On November 1, 2018, the U.S. Food and Drug Administration (FDA) began investigating an outbreak of *E. coli* O157:H7 infections across the U.S. and Canada. There were 62 reported cases across 16 states where 25 people were hospitalized. After further investigations The FDA and the Centers for Disease Control and Prevention (CDC) determined that infected romaine lettuce was the cause of this illness outbreak and a massive recall was initiated. The FDA and the CDC conducted epidemiological traceback analysis to determine the source of the contaminated produce and identified the outbreak strain of *E. coli* O157:H7 in sediment collected from an agricultural reservoir in California (CDC, 2019; FDA, 2019).

The two primary methods of *E. coli* O157:H7 transmission are through food and water. The most frequent location for water contamination occurs in runoff farms from manure applications, irrigation waters, and/or interactions with waterfowl (Ishii et al., 2007). The contamination of surface waters from *E. coli* and other fecal bacteria is a function of multiple variables including the fecal deposition site, size and quantity of livestock, locations of the livestock, livestock fecal deposits in relation to distance from waterbodies, and survival of bacteria from the time of deposition and surface runoff events (Larsen et al., 1994). Livestock grazing is frequently identified as a contributor of fecal coliforms, which has resulted in required measures by the U.S. government to reduce *E. coli* occurrences and improve surface water quality (TCEQ, 2007, 2008). The direct relationship between livestock grazing and *E. coli* concentrations in runoff and surface water bodies has been linked to either direct deposition of fecal matter or subsurface and surface flow (Doran and Linn, 1979; Doran et al., 1981; Gary et al., 1983; Tiedemann et al., 1987; Donnison et al., 2004). Surface runoff is the main method for the transport of *E. coli* into streams due to its attachment to soil particles (Collins et al., 2005). Therefore, best management practices (BMPs) (e.g, vegetated filter strips; wetlands) have been recommended for grazing operations to reduce stream impairment due to *E. coli* and other fecal coliform bacteria (Wagner et al., 2012).

However, recent findings have indicated avian populations may significantly affect *E. coli* exceedance occurrences. To date, little is known for predicting the *E. coli* occurrence in waterways, which leads to challenges for identifying appropriate and effective prevention and treatment practices (De Brauwere et al., 2014; Lothrop et al., 2018). Further, the ubiquitous occurrences of *E. coli* exceedances throughout the U.S., particularly in water-limited regions such as the Plains, accentuates the urgency of identifying fate and transport patterns of *E. coli* in waterways. A better understanding of the fate and transport of *E. coli* in agroecosystems would improve recommendations for monitoring practices and BMPs for water quality improvements in adjacent and downstream waterbodies. Therefore, the primary objectives of this study were to: (1) Determine the load of *E. coli* during storm events in a hydrologic controlled stream situated adjacent to a livestock grazing operation and centered in the fly zone for avian migration in the Plains and (2) Identify trends between *E. coli* concentrations in water, grazing rotations, and avian migration patterns.

Materials and Methods

Site Description

The study location was within the United States Meat and Animal Research Center (USMARC) near Clay Center, Nebraska. The site was once a Naval Ammunition Depot (NAD) utilized to manufacture and store large ammunitions during World War II. However, Congress approved legislation in 1964 that began the transfer of this NAD to the United States Department of Agriculture, thus creating USMARC. Development of USMARC began in the spring of 1966 on 14,200 hectares (34,000 acres) near Clay Center, Nebraska. Groundwater contamination was found on the USMARC property in the mid-1980s, which resulted from munitions manufacturing activities of the former NAD. Two plumes of groundwater contamination were identified. The U.S. Army Corps of Engineers (USACE) developed and implemented a groundwater remediation strategy for the plumes involving the installation of multiple extraction wells and a water treatment facility, with the opportunity for agricultural reuse of the treated groundwater (USACE/EPA, 2010).

In full collaboration with USACE and the Little Blue Natural Resources District of Nebraska, USMARC has worked since 2010 to develop and implement the plan for groundwater remediation and water reuse on USMARC property. The plan has involved placing a groundwater treatment facility and all associated piping on USMARC property. Construction of an air stripping treatment plant, extraction wells, and pipeline system for the north plume were completed, and the water remediation plant began operation in April 2013. Construction of the extraction wells, well houses, and pipeline for the southern plume were completed in the fall of 2014. The wells remove an estimated 14,000 L m^{-1} on a continuous basis. Beneficial reuse of the treated water includes the irrigation of USMARC feed crops and pastures along with discharge of remediated water to an existing stream, which flows throughout the USMARC property (Figure 3.1). Nine grade control structures (GCS) retain water across the site to increase percolation of the treated water back into the ground and to also help prevent erosion from high-flow storm events.

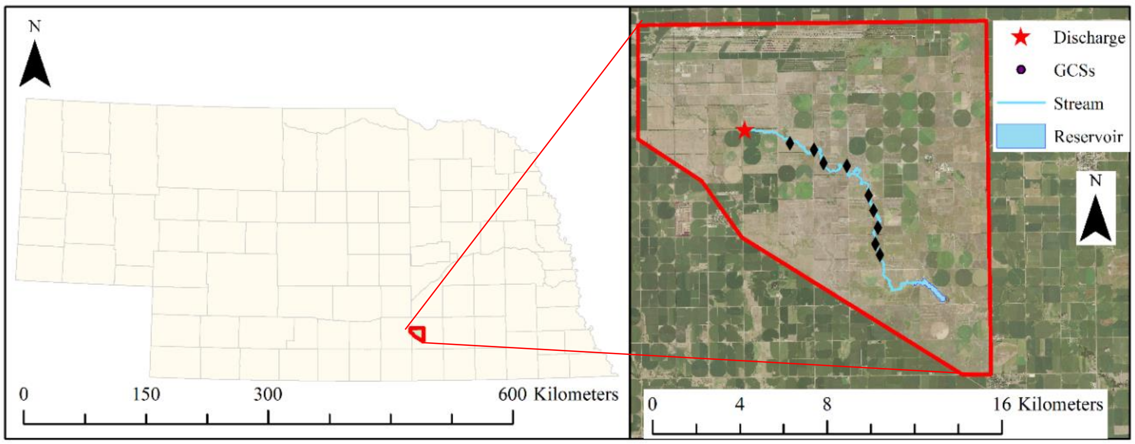

Figure 3.1: *United States Meat Animal Research Center location within Nebraska, USA*

Hydrologic Monitoring

Hydrologic monitoring was conducted at five locations throughout the study site using five portable surface water samplers (ISCO, Teledyne, Lincoln, NE, USA; Figure 2). ISCOs were placed below three of the GCSs, at the Discharge, and at the reservoir to determine *E. coli* loads moving through the stream system during the summer and fall of 2018. Each ISCO was outfitted with a pressure sensor, which recorded water depth every five minutes from April 20th, 2018 through October 25th, 2018. A few water depth readings were lost due to equipment malfunction and supplemented by additional HOBO water depth loggers (Onset HOBO, Bourne, MA, USA), which were installed next to the ISCO samplers. Each ISCO was also outfitted with ISCO 674 rainfall tipping buckets, which were compared to dedicated manual rain gauges located across the USMARC site. Flow rates were calculated using the Kindsvater-Carter equation suppressed rectangular, sharp-crested weir,

$$Q = (0.4000\left(\frac{H}{P}\right) + 3.220)(L - 0.003)(H + 0.003)^{3/2},$$

where Q = flowrate (cfs), H = water level (ft), P = height of the weir (ft) and L = length of the weir crest (ft). All flowrates were then converted into metric units. This equation calculated the flowrates of GCS1, GCS2, and GCS5 throughout the study period, while the Discharge flowrate was taken directly from the recorded flowmeter from the wet well pump.

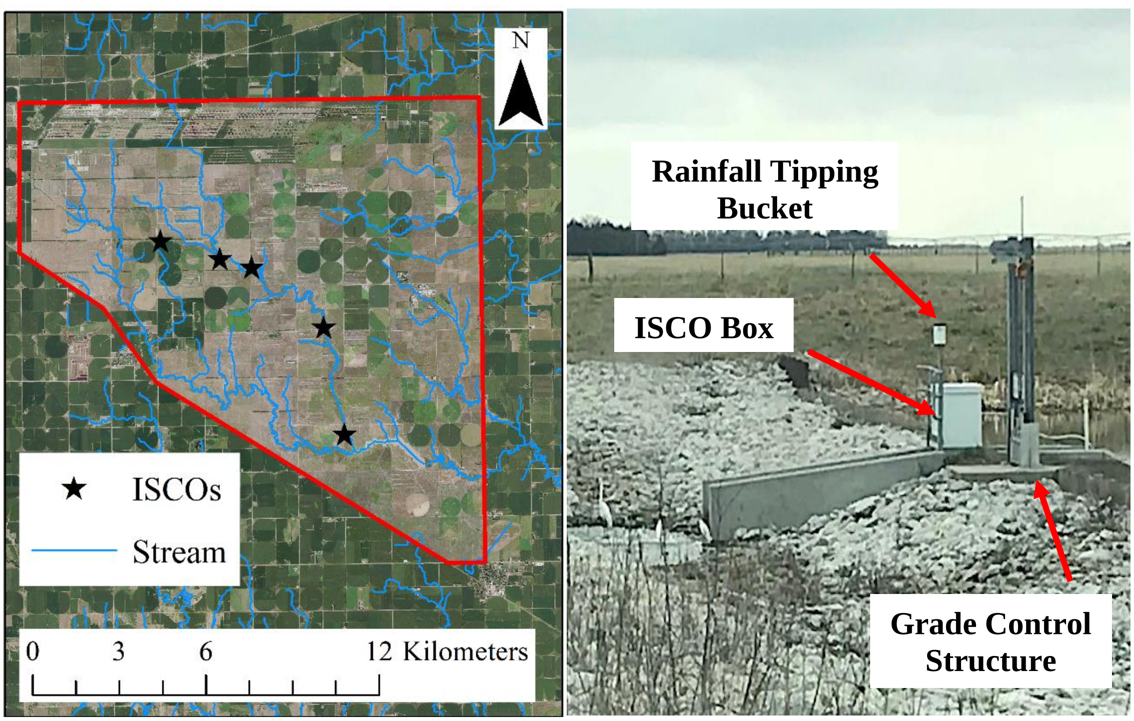

Figure 3.2a and 3.2b: *(left) ISCO locations across the USMARC site; (right) ISCO 6712 water sampler setup*

Water Quality Monitoring

There were a total of five ISCO portable water samplers implemented in this study, which included (Figure 3.2a): the outflow from the groundwater treatment systems (Discharge), the first grade control structure (GCS1), the second grade control structure (GCS2), the fifth grade control structure (GCS5), and the reservoir below the 9th grade control structure referenced as 9Res. ISCO rain gauges were configured for each ISCO to initiate event-based water sampling. The ISCOs utilized a peristaltic pump to draw water from the stream to one of 12 1,000 mL glass bottles. The first six glass bottles were designated “Group A” and were sampled more frequently (1 sample/30 minutes) in order to catch the first flush of total *E. coli* following a rainfall event. The remaining six glass bottles were designated “Group B” and were sampled less frequently (1 sample/hour) in order to collect total *E. coli* concentrations once stream flow returned to baseflow. The ISCO samplers were programmed to take water samples based on event-based criteria. The program criteria was designed to begin sampling water immediately after rainfall rates reached 1.3 mm hr^{-1}. After the first sample was collected, the frequency followed the designated group frequencies.

Water Quality Analysis

Samples were analyzed within 24 hours of collection to ensure the survival of the bacteria. Once the samples were collected, they were transported back to Lincoln, NE to be analyzed and enumerated. Total *E. coli* concentrations were determined with the IDEXX 97-well Colilert®-18/Quanti-Tray®/2000 analysis. This method is more accurate than previously used methods and takes less time for incubation and it does not require a confirmation test (Sartory and Vandevenne, 2009). β-galactosidase was used to detect coliform bacteria, while β-glucuronidase was used for the detection of total *E. coli*. The selective growth medium containing the enzyme substrates was added to each water sample and divided into a series of reaction wells, 49 large reaction wells and 48 small reaction wells. After incubation, if coliforms are present, the wells would change to a yellow color. If any *E. coli* was present within an individual reaction well, the reaction well would fluoresce under a black light. Once the number of fluoresced large and small wells were counted, a chart provided by IDEXX is used to estimate the most probable number (MPN) of *E. coli* bacteria per 100 m L^{-1} of sample. Additionally, water samples were analyzed for nitrate-N (NO_3-N) and phosphate-P (PO_4-P) using an AQ2 (Seal Analytical; Mequon, Wisconsin) with the EPA methods EPA-103-A Rev 10 and EPA-127-A Rev 8, respectively.

The exceedance probability was used to evaluate the likelihood of *E. coli* exceedances at each of the monitoring locations. Exceedance probability is a method of ranking measured environmental concentrations to show how likely future measurements will exceed the previously recorded concentrations. The equation to calculate exceedance probability is:

$$P = 100\% * \frac{m}{(n+1)},$$

Where P is the exceedance probability percentage, m is the rank of the concentration value, and n represented the total number of concentration values used. The concentrations used for the produced exceedance probability curves included five recorded storm events for *E. coli* MPN concentrations, NO_3-N concentrations, and PO_4-P concentrations.

Cattle Grazing Rotations

USMARC has 790 individual pastures of which cattle are rotated in an effort to control manage pasture forage. Grazing records, including the grazing dates and pasture locations was used to identify potential bovine interactions with the stream during studied storm events. The number of pastures were filtered down to 92 to include only those pastures that drain into the our study watershed and within 50 meters of the stream (Figure 3.3). The assumption for this constraint is that when cattle are in closer proximity to the stream, there is a higher probability of *E. coli* delivery and contamination. Berry et al., (2014) found that *E. coli* bacteria was recovered in 3.5% leafy green crops at a distance of 60 meters from a cattle feedlot but was only 1.8% of leafy green crops at 180 meters. Further, for this study, 50 meters was chosen for the proximity limit to the stream in an attempt to identify how close proximity of cattle to the stream, affected the total *E. coli* water concentrations. The number of cattle present was also determined based on the number of Animal Units (AUs), which varies on the overall development of the herd. The animal unit equivalents are a method of standardizing the size of individual bovine based on weight and development that assists in normalizing other factors that are related to the number of head of grazing cattle (Manske, 1998). Therefore, AUs were used instead of the number of head of cattle in this study as a way of normalizing the presence of cattle within each catchment.

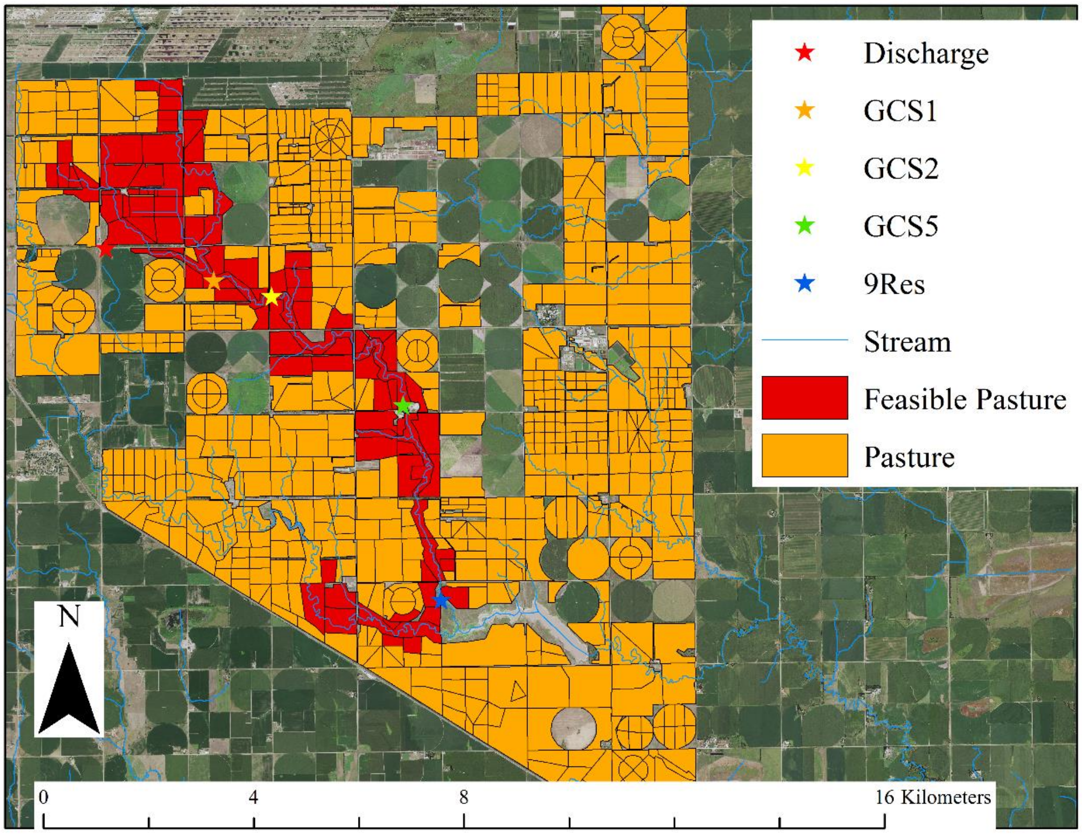

Figure 3.3*: United States Meat Animal Research Center pastures and the selected pastures for this study*

Time Lapse Bird Cameras

Three different time lapse cameras (TRLcam; Walton, NE) were installed to record the presence of the waterfowl on site. Two cameras were placed near the downstream reservoir, where majority of the migratory waterfowl visited the site (Figure 4a) and one at the smaller lake behind GCS1 (Figure 3.4b). In order to compare the bovine presence to the migratory waterfowl presence, areal densities were calculated using ArcMap. The lake area of the West Lake, East Lake and GCS1 camera coverage were 11.6, 19.9 and 4.9 hectares, respectively. The method of estimating the number of birds in each picture was to break the flock into units of 10, or 100 birds, and then estimate the number of these units within the whole flock in each picture. This method is common for estimating flock size

(USFWS, 2019). The number of birds present were estimated on a weekly basis throughout the study period, adding all usable pictures, taken at hourly intervals from sunrise to sunset.

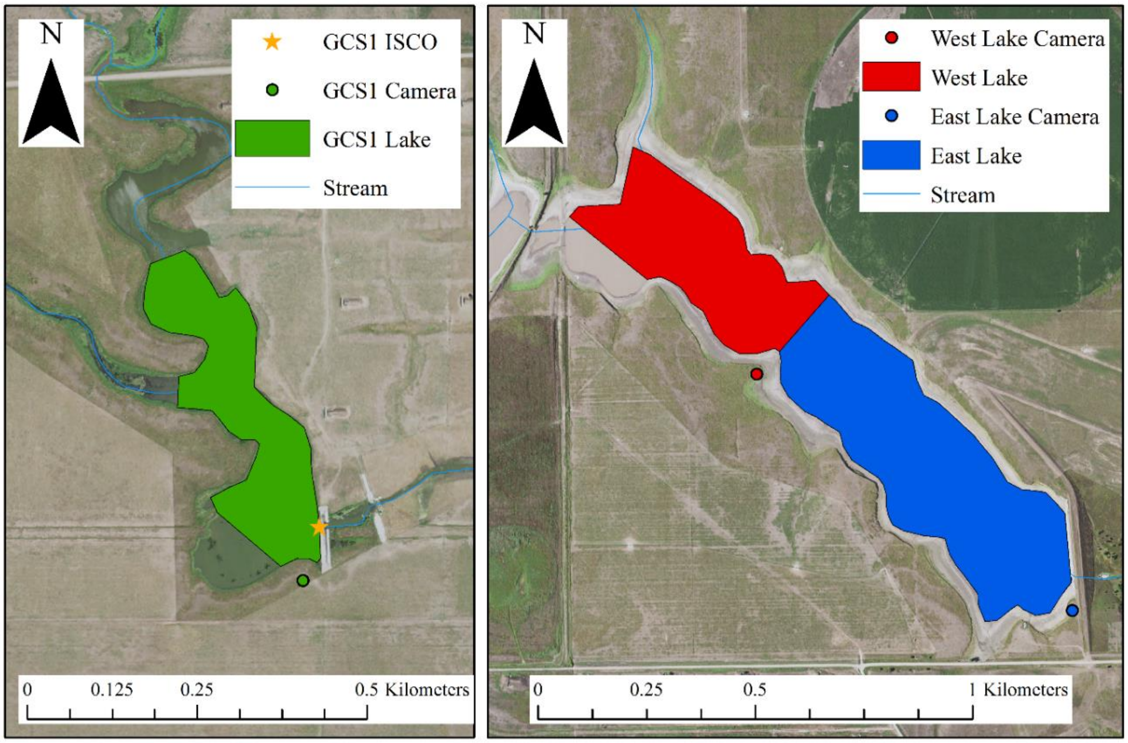

Figure 3.4a and 3.4b*: United States Meat Animal Research Center camera locations and lake coverage for grade control structure 1 (left) and the reservoir (right)*

Multiple Least Square Regression

E. coli data was normalized by log transformation and assessed using a two-way ANOVA with season (n=2) and source (n=2). Relationships between *E. coli* concentrations, bovine density, waterfowl density, and season were tested using simple and multiple linear regressions. Potential source predictions were performed to determine the primary source of *E. coli* concentrations using p value and adjusted R^2. Reported significance was determined at $\alpha = 0.05$. All statistical analyses were completed in JMP 14 (SAS Institute Inc., 2019).

Results

Measured Hydrology

A unique quality of this project was that the water at the Discharge was constant throughout the year, which enabled the change in water quantity within the stream to be assessed across the system. During the experiment, the Discharge pipe of the treated groundwater source water was metered and manually controlled by the facility managers. Each GCS was designed to reduce erosion and allow percolation of the treated water back into the groundwater by slowing down the flow of water. Therefore, 1 to 4 0.3 m stop logs were placed in the GCSs; 1, 2, 4, 5, and 6 to retain localized water and were gradually taken out to release the water once more water was available to be stored in the downstream reservoir. However, there are some GCSs that do not have stop logs, 3, 7, 8, and 9, making it difficult to quantify the volumetric flow rate leaving each GCS. The location of the 9Res ISCO was essentially the beginning of the reservoir and did not have a weir so discharge was not calculated.

Accumulated flow from the 4 of the 5 monitoring locations are found in Figure 3.6. The red line represents the accumulated flow of the source of the water from the Discharge, which was consistent with the daily flowrate except for two periods (shaded on the graph). The first 10 day period was for maintenance from June 6, 2018 to June 16, 2018, where the flow was reduced from 14,000 L m^{-1} to 8,700 L m^{-1}. The second 2 day period, which occurred from September 4, 2018 to September 6, 2018, was due to a downstream flood risk, where the pump was completely turned off reducing the flowrate from 14,000 L m^{-1} to 0 L m^{-1}.

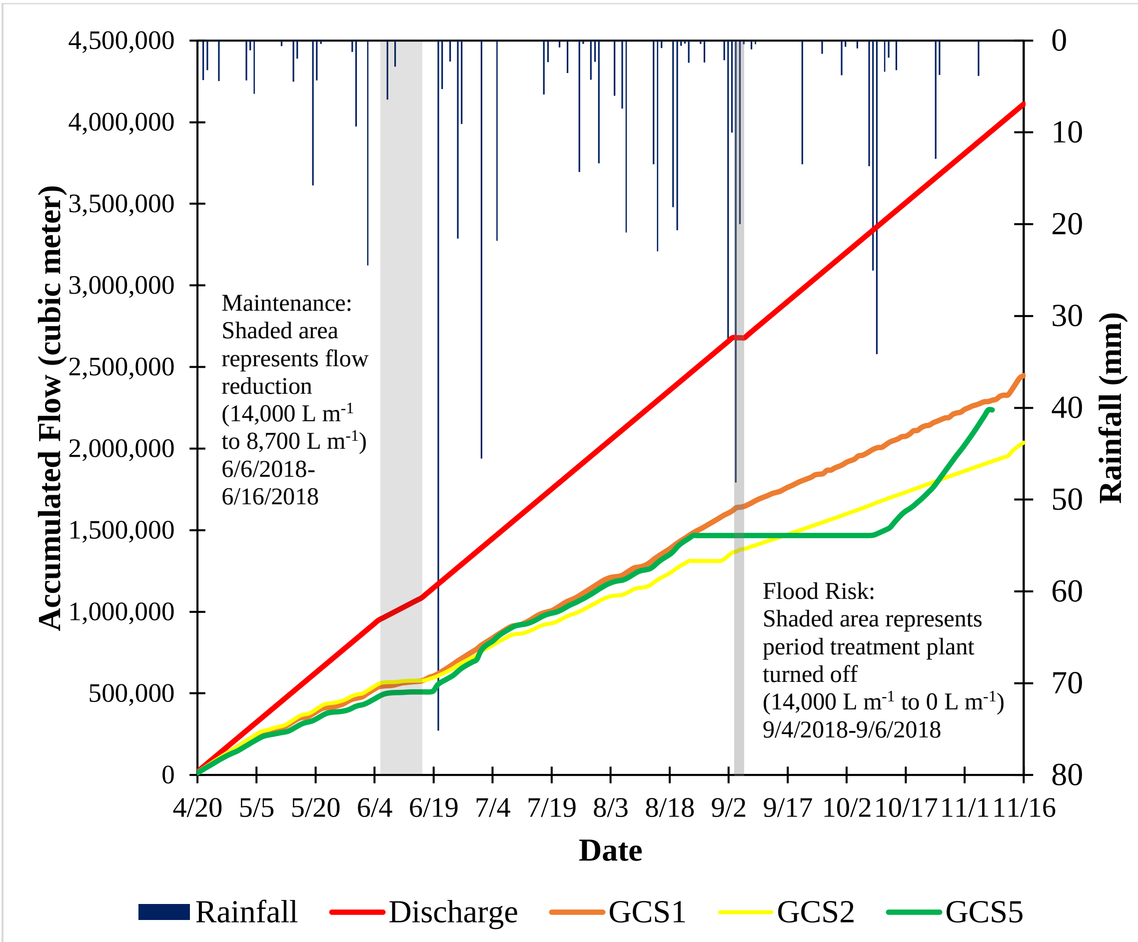

Figure 3.6*: Observed flow accumulation at various locations at the United States Meat Animal Research Center during 2018*

Water Quality Load Assessments

Exceedance probabilities were determined for *E. coli*, NO_3-N, and PO_4-P concentrations recorded for the five storm events (Figures 3.7, 3.9, and 3.10). *E. coli* and PO_4-P concentrations increased as water moved through the system, while NO_3-N concentrations decreased through the system.

The EPA's fresh water quality criteria for *E. coli* is that any one grab sample must not exceed 235cfu/100mL (Figure 3.7). The Discharge rarely ever recorded any *E. coli* because it's only source of water was the treated groundwater. However, GCS1 exceeded this limit ~26% of the time, GCS2 exceeded this limit ~40% of the time, GCS5 exceeded this limit ~75% of the time, and 9Res exceeded this ~85% of the time. Total *E. coli* concentrations of each storm were averaged for each location in the system, which showed that total *E. coli* concentrations usually increased as water flowed down the stream following rainfall events (Figure 3.8).

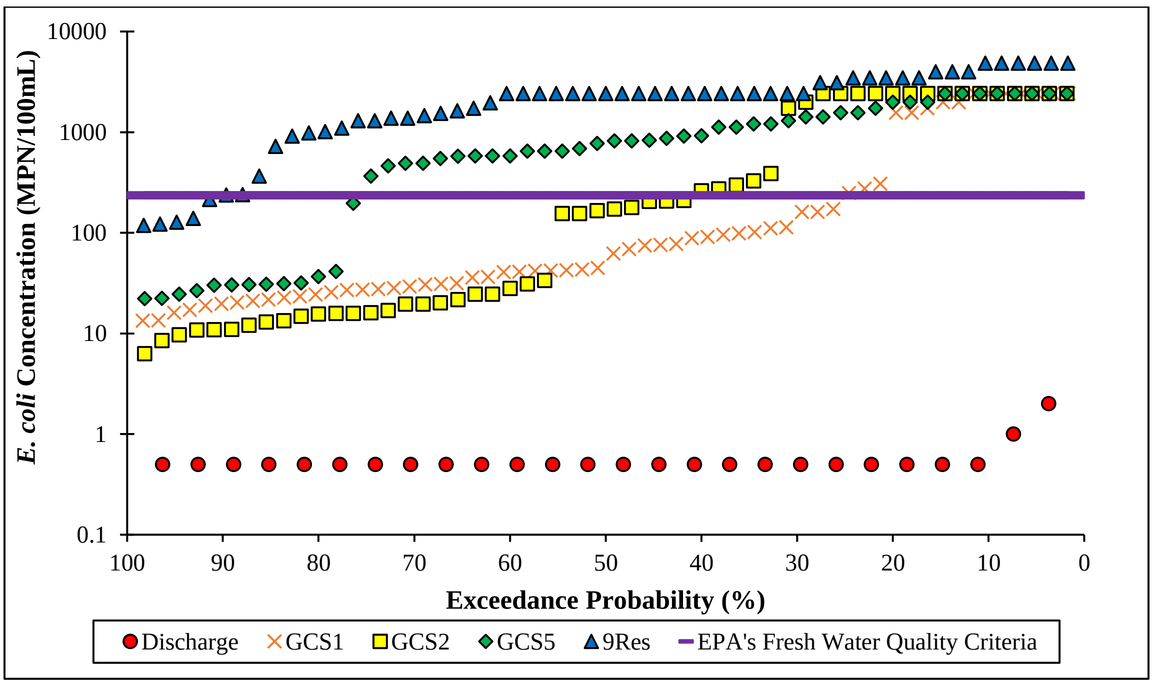

Figure 3.7: *Exceedance probability of E. coli surface water concentrations during the growing season*

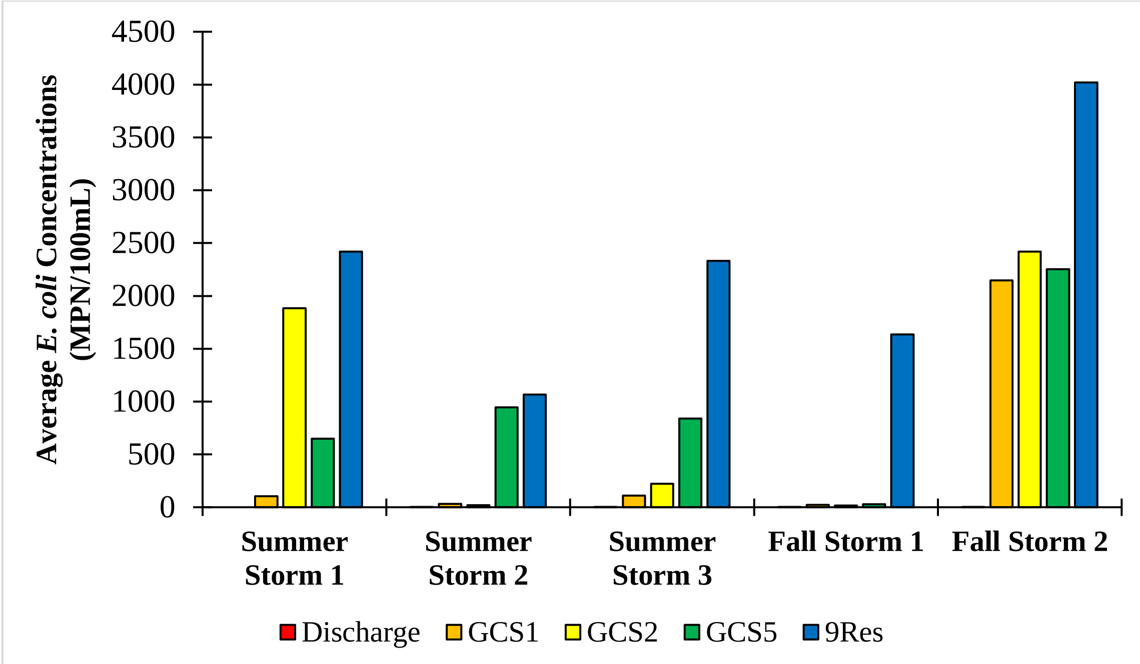

Figure 3.8*: Average E. coli concentrations for each storm event during the study period for each ISCO location*

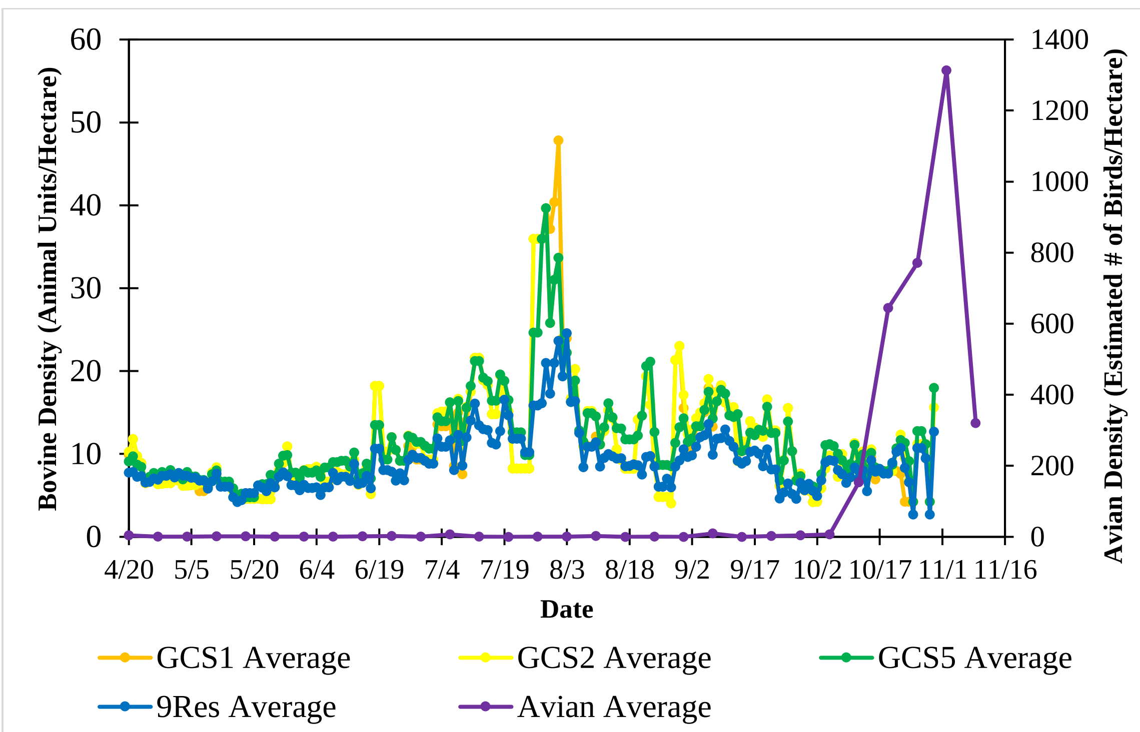

Figure 3.9*: Areal densities of cattle and waterfowl throughout the study period*

The exceedance probability curve for the observed NO_3-N concentrations exhibited in the stream acted as a NO_3-N "losing" stream system, where NO_3-N concentrations continued to decrease as water flowed from the Discharge through the system to 9Res. This phenomenon is common among Nebraska groundwater discharged streams. The groundwater often has the higher NO_3-N concentrations, and as the water flows through the system, the plants or floating algae uptake the available NO_3-N. Or another possible process would be that when the NO_3-N gets to the GCS reservoirs, it can undergo anoxic conditions and denitrification can occur.

In contrast, PO_4-P concentrations "gained" throughout the system, where PO_4-P concentrations began low from the Discharge and increased as water flowed through the system, similar to *E. coli*. This is evident when considering the sediment load throughout the system. Groundwater pumped to the discharge has low turbidity and as the water flows through the system, it accumulates an increasing amount of sediment, making the water more turbid. Both PO_4-P and *E. coli* tend to bind to soil particles due to their surface charge, often indicating the presence of PO_4-P and *E. coli* in more turbid waters.

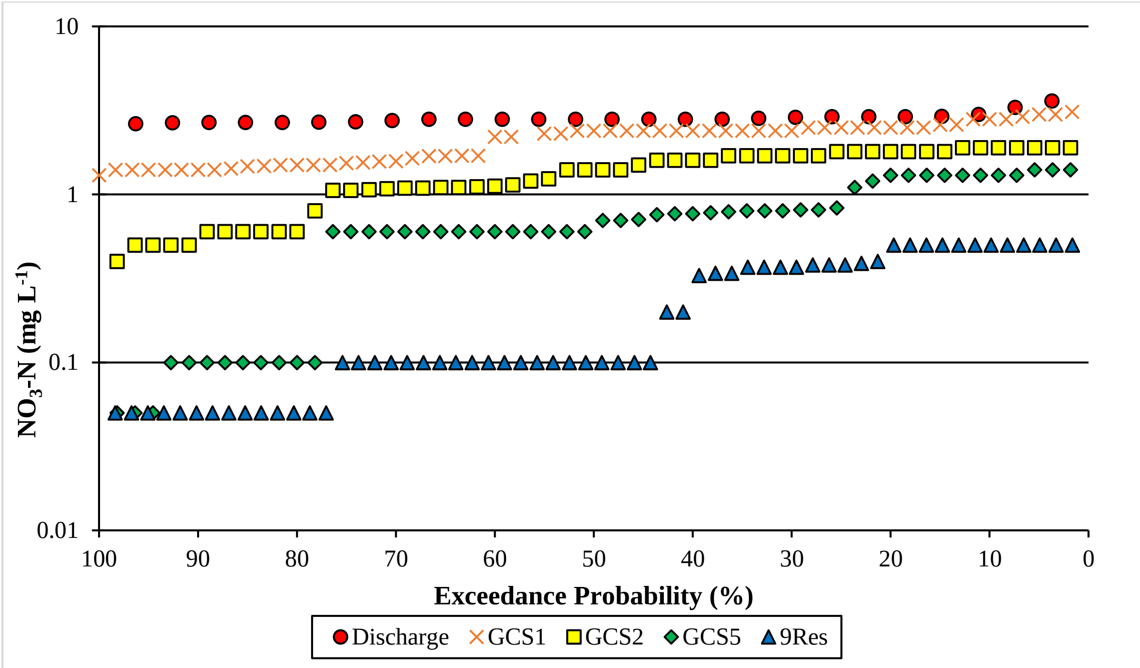

Figure 3.10: *Exceedance probability of NO_3-N surface water concentrations during the growing season*

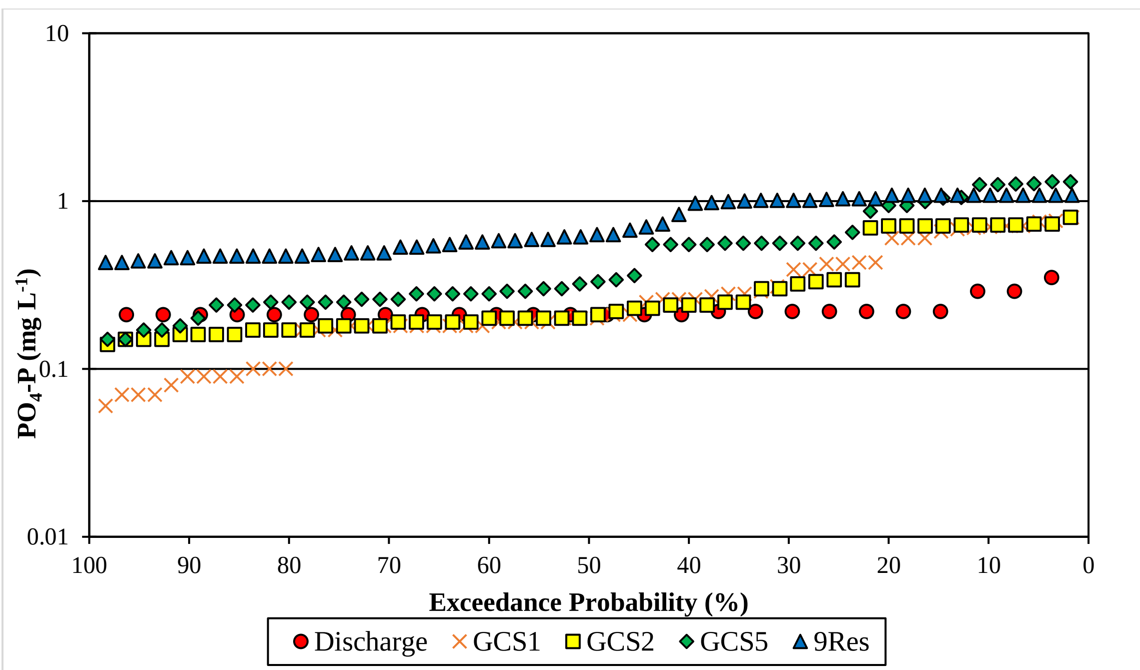

Figure 3.11: *Exceedance probability of PO_4-P surface water concentrations during the growing season*

Source Tracking

Few studies have identified the magnitude of migratory waterfowl on the observed *E. coli* concentrations within the water (Gorham and Lee, 2016; Elmberg et al., 2017). However, several studies have attempted to quantify bovine grazing practices influence on *E. coli* concentrations within watersheds. Larsen et al., (1994) found that the contamination of surface waters from *E. coli* and other fecal bacteria was a function of the characteristics of the fecal deposition site, size and number of cattle, locations of the cattle and their fecal deposits in relation to the water bodies, and the survival of bacteria from the time of deposition and surface runoff events. Therefore, in this study the *E. coli* concentrations for all five storm events were evaluated based on the proximity of the number of grazing cattle within 50 m of the stream, the relationship between the number of grazing cattle within the catchment and the average *E. coli* concentration in water during a storm event (Figures 3.12, 3.13, and 3.14).

E. coli concentrations had a strong correlation with the increasing accumulation of bovine on the pastures throughout the growing season (Figure 3.12, and 3.14), with a stronger when cattle were present on the fields adjacent to the stream on the day of the rainfall event. These observations are similar to past *E. coli* studies focused on cattle (Larsen et al., 1994; Wagner et al., 2012; Derlet et al., 2012). There was no evident relationship between the accumulated number of recently grazing cattle within the catchment between storm events of greater than 2.54 cm and the average concentration of *E. coli* observed for each storm event (Figure 3.13). Similarly, a weak relationship was observed between average *E. coli* surface water concentrations and observed avian populations the day before and during the rainfall event (Figure 15).

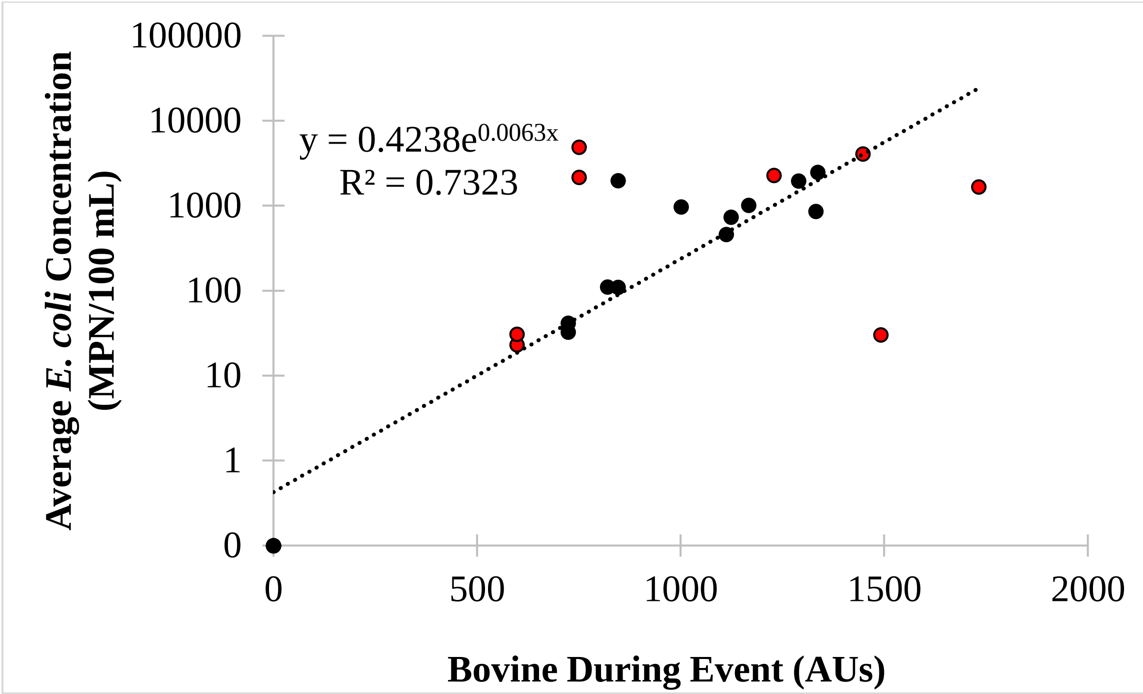

Figure 3.12*: Average E. coli concentration with respect to the number of grazing cattle within the 50 meters of the stream on the day of the rainfall event. *Red data points indicate fall samples and black data points indicate summer samples.*

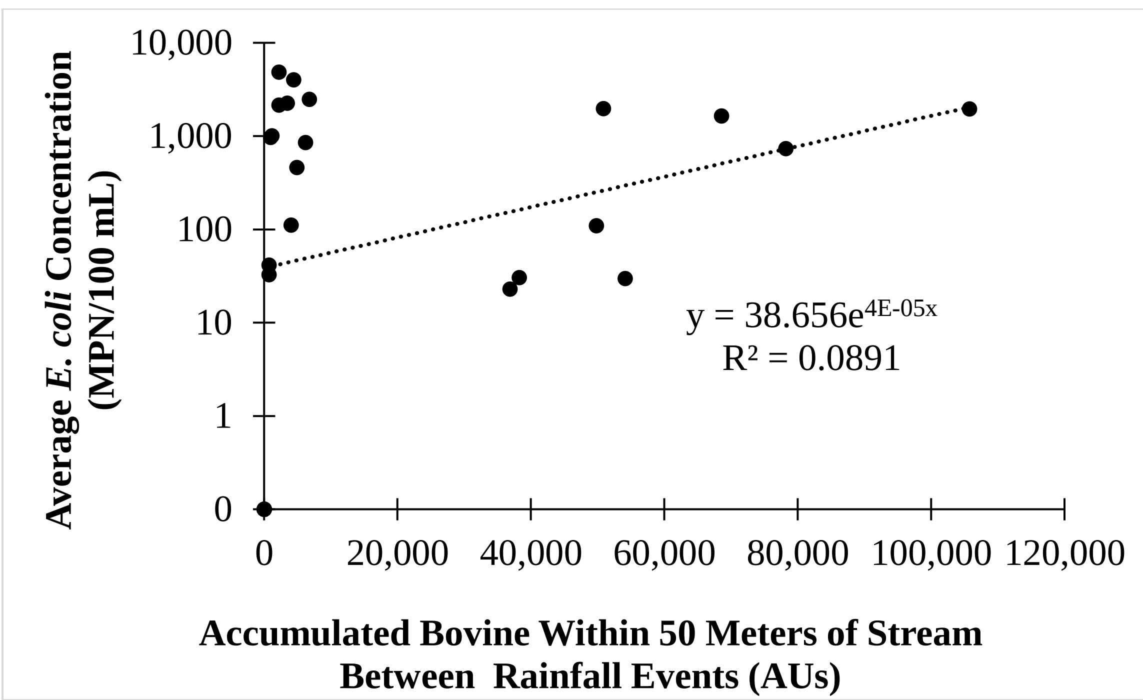

Figure 3.13*: Average E. coli concentrations in water with respect to the accumulated number of cattle grazing within 50 meters of the stream, in between rainfall events of 2.54 centimeters or greater.*

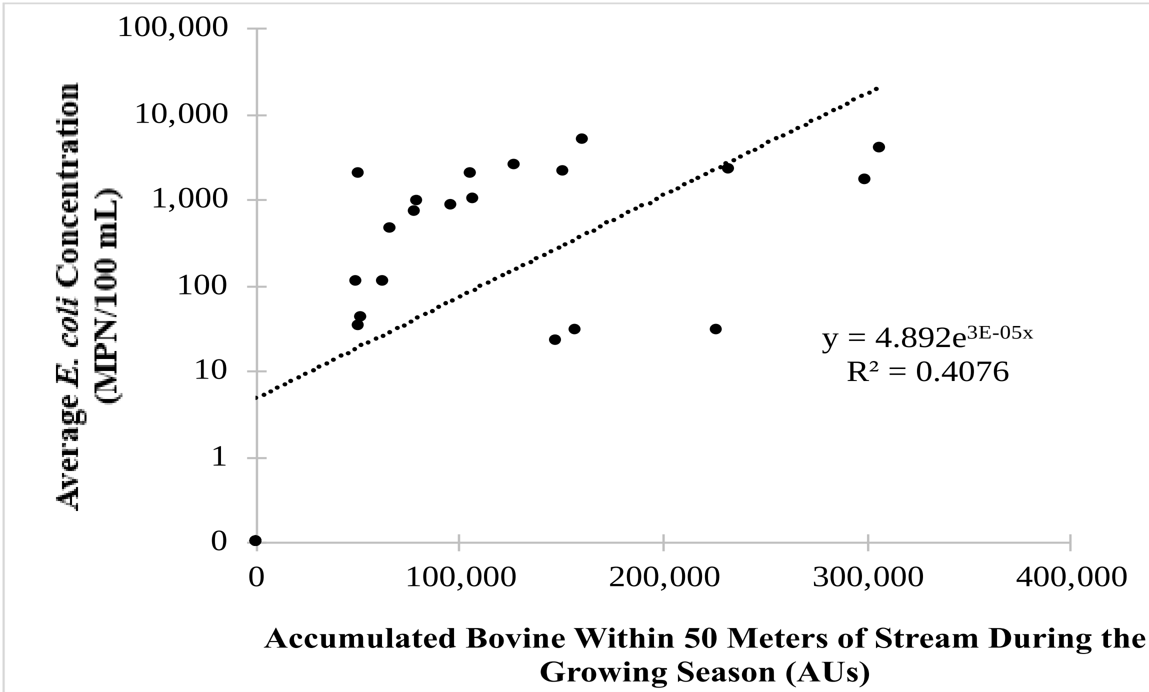

Figure 3.14*: Average E. coli concentrations with respect to the accumulated number of cattle grazing over the growing season within 50 meters of the stream.*

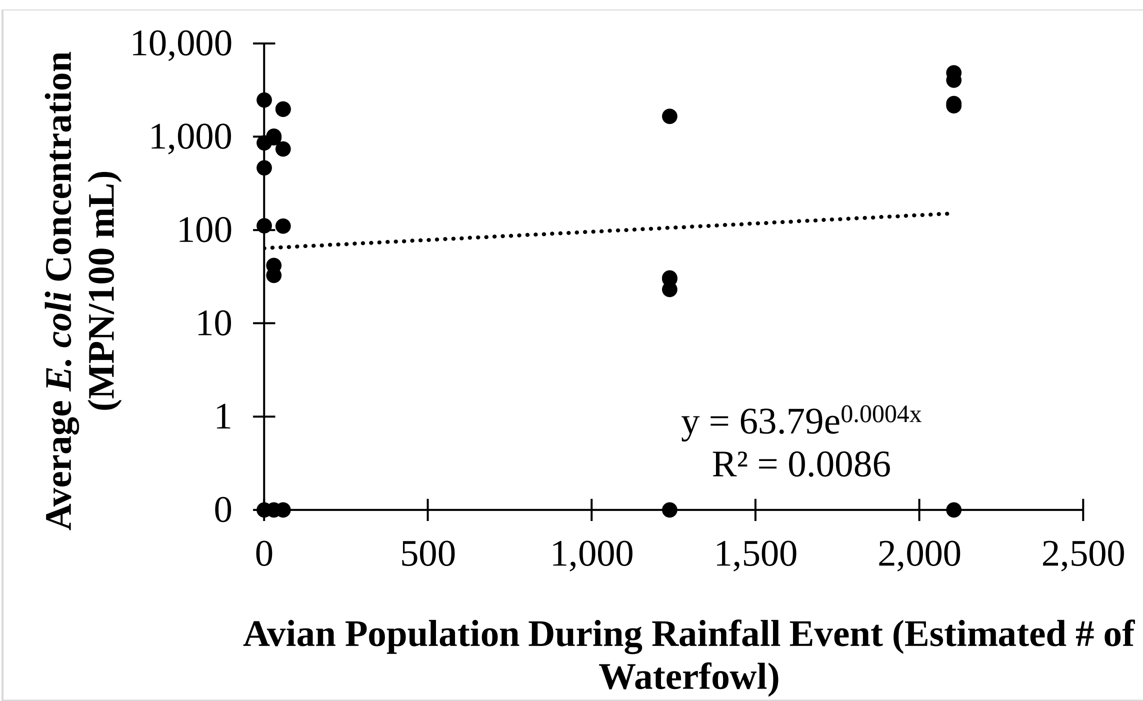

Figure 3.15*: E. coli concentrations with respect to the number of present migratory waterfowl on site the day of the rainfall event.*

Strong correlations were observed between bovine presence within 50 m of the stream and *E. coli* concentrations; however, outliers were typical during the fall when the waterfowl arrived. An ANOVA test was completed to further determine seasonality effect. Bovine presence both the day of and within 30 days was found to be significant when evaluating the entire season of *E. coli* concentrations ($p<0.0001$), while waterfowl during the rainfall events was not significant. However, presence of waterfowl on the day of the event and bovine presence 30 days or more prior the event both were found to be significant ($p=0.0005$ for bovine and $p=0.0314$ for waterfowl). Further examination of the dataset seasonally revealed that bovine was the primary source that correlated with *E. coli* concentrations during summer events (p-value <0.0001), while waterfowl was the primary source *E. coli* concentrations correlated with during the fall events ($p=0.0422$).

Discussion

The primary contributing source of *E. coli* to the stream system was impossible to definitively determine without completing additional microbial assays. However, the trends in the *E. coli* concentrations, along with the detailed pasture grazing information and estimated number of migratory waterfowl provide new insight regarding the impacts of multiple species to the variability of total *E. coli* water concentrations throughout the growing season. Additionally, the statistical analysis completed in this study showed that the bovine presence was the main factor in predicting total *E. coli* concentrations when examining just the summer rainfall events. However, when examining just the fall events, the waterfowl presence was the main contributor to the total *E. coli* water concentrations.

Overall the bovine presence within fifty meters of the stream was significantly the main predictor of *E. coli* when examining the entire season.

Primary *E. coli* sources examined in this study were from rotational cattle grazing and migratory waterfowl, which interact with surface water in completely different transport scenarios. Cattle contribute significant quantities of manure, and *E. coli* has been reported to survive for up to 77, >226, and 231 days in manure-amended soil held at 5, 15, and 21℃, respectively (Jiang et al., 2002). *E. coli* can persist through a variety of climatic conditions through various agricultural media, and has been reported to survive for at least 245 days in cattle water troughs (LeJeune et al., 2001), and on common farm surfaces such as galvanized steel and wood posts (Williams et al., 2005). It has been reported that large rainfall events have had significant impacts on surface water total *E. coli* concentrations. Kleinheinz et al., (2009) reported six out of eight beach water *E. coli* concentrations in Door County, Wisconsin showed a large impact from significant rainfall events (> 5 mm in 24 hour period). Therefore, waterbodies are more susceptible in regions where cattle are closer and may have direct access to the stream (Nagels et al., 2002; Line, 2003; McKergrow et al., 2003; Muenz et al., 2006; Vidon et al., 2008), similar to the conditions presented in this study. There is also the natural background contribution of bacteria to surface water runoff from local wildlife (mice, rabbits, raccoons), making the contributions harder to definitively pinpoint (Doran et al., 1981).

Best management practices to reduce *E. coli* contributions to surface water include limiting cattle access to streams (Vidon et al., 2008), reducing the number of cattle grazing near a stream (Gary et al., 1983), and implementing vegetative filter strips along stream corridors (Fox et al., 2011). Vegetative filter strips along stream banks remove *E. coli* from surface water runoff similarly to phosphorous, where reported high correlations between *E. coli* concentrations and suspended sediments (Anderson and Rounds, 2003). Filter strips remove *E. coli* up to 99%; however, efficiency of the vegetative filter strips significantly reduce as runoff increases (Tate et al., 2006). *E. coli* typically binds to soil particles of < 2 μm, implying an unattenuated effect during overland flow transport. However, *E. coli* still binds to larger sediment particles which are able to be physically filtrated and removed from the water column in the filter strips (Muirhead et al., 2006).

Management of *E. coli* contributions by avian species present a more challenging scenario. The key method of *E. coli* contamination from migratory waterfowl is from direct fecal deposition into the waterbody, due to a majority of their time spent in water rather than on land (Lickfett et al., 2018). One of the main methods of inactivation of the bacteria *E. coli* is through ultraviolet exposure (Vermeulen et al., 2008; Davies-Colley et al., 1994); therefore, increasing surface area and reducing water depth of these retention areas along the stream is expected to enhance *E. coli* concentration reduction. However, future research is needed to assess this management practice on the overall ecology of the waterbody. Besides physical alterations of the streams, in-stream removal processes are another recommendation that have the potential to reduce *E. coli* concentrations within the water column. For instance, wetlands, specifically designed with aquatic macrophytes, have been found to efficiently reduce the concentration of *E. coli* through die-off and possibly microbial competition or protozoa predation (Karim et al., 2008; Hickey et al., 2018; Saeed et al., 2014). Knox et al., (2007) found that wetlands removed *E. coli* at a range of 33%-91% with an average removal of 73%.

Conclusion

A complex combination of bovine density and waterfowl migration patterns significantly impacted measured stream *E. coli* concentrations in the summer and fall rainfall events. During the summer season, bovine density within 50 m of the stream up to 30 days prior to rainfall events impacted *E. coli* exceedances within the stream corridor. However, waterfowl increased *E. coli* concentrations, specifically in slow flowing portions of the stream, where birds congregated during the fall season. *E. coli* concentrations accumulated as water moved along the stream corridor regardless of season. The downstream reservoir had exceedance probabilities above the EPA freshwater criteria >85% of the growing season following rainfall events. Recent illness outbreaks of pathogenic *E. coli* originating from irrigation water demonstrate that implementation of best management practices is critical for preventing future outbreaks. Additionally, testing the irrigation water prior to application should be considered in the future. Further research is needed to determine in-situ and adjacent to stream BMPs to minimize *E. coli* contamination in irrigation reservoirs.

CHAPTER 4: OVERALL CONCLUSIONS OF THESIS, RECOMMENDATIONS, AND FUTURE WORK PROPOSALS

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Conclusions

Based on the potential health and ecological implications of atrazine, NO_3-N, and *E. coli* being present in surface water, further investigation is needed to identify "hot" times and "hot" spots in in the Midwest. Further, based on results from this project, the identification of primary contributors is critical for placement of preventative measures (e.g., best management practices). Therefore, the following objectives were evaluated during this project:

1. Identify watersheds across Nebraska that were at risk for exceeding nitrate-N and atrazine maximum contaminant limits (MCLs) in surface water (Chapter 2)*;*
2. Determine the specific times in the year where risks were greatest (Chapter 2)*;*
3. Determine the load of *E. coli* during and following storm events at a continuous rotational livestock grazing operation in central Nebraska (Chapter 3)
4. Identify trends between *E. coli* concentrations in water, cattle grazing rotations, and avian migration patterns (Chapter 3).

Findings to assess these objective formed the following conclusions

Objective 1: The risk factor associated with NO_3-N exposure remained consistent in its spatial distribution throughout this study, while the atrazine risk was observed in regions of Nebraska with high corn production, which resulted in these regions subsequently resulting in higher risks for dual exposure to atrazine and NO_3-N. The dual risk factors were highest in the southeastern region of Nebraska, primarily due to the increased risk of atrazine exposure.

Objective 2: Atrazine was found to be susceptible to the spring flush effect, while surface water NO_3-N concentrations were consistent throughout the year. During years with dry springs, the risk of exposure to atrazine and NO_3-N were reduced.

Objective 3: The downstream reservoir had exceedance probabilities above the EPA freshwater criteria >85% of the growing season following rainfall events.

Objective 4: A complex combination of bovine density and waterfowl migration patterns significantly impacted measured stream *E. coli* concentrations in the summer and fall rainfall events. During the summer season, bovine density within 50 meters of the stream up to 30 days prior to rainfall events impacted *E. coli* exceedances within the stream corridor. However, waterfowl increased *E. coli* concentrations, specifically in slow flowing portions of the stream, where birds congregated during the fall season. *E. coli* concentrations accumulated as water moved along the stream corridor regardless of season.

Recommendations

Nitrate/Atrazine Project (Objectives 1 and 2)

- Increase use of vegetated filter strips along agricultural streams
- Implement *in situ* treatments to mitigate atrazine and nitrate concentrations such as floating treatment wetlands or vegetated ditches
- Locate watersheds where best management practices would be most effective
- Implement automated autonomous weed sprayers that could reduce the total amount of atrazine used

***E. coli* Project (Objectives 3 and 4)**

- Increase use of vegetated filter strips along agricultural streams
- Implement *in situ* treatments to mitigate *E. coli* concentrations such as floating treatment wetlands or vegetated ditches
- Fence of streams near animal grazing operations
- Move cattle grazing further upstream when rainfall is imminent
- Use irrigation water from more upstream sources to reduce possibility of *E. coli* contamination

Future Work

Nitrate/Atrazine Project (Objectives 1 and 2)

- Apply or modify dual risk methodology to other contaminant mixtures
- Apply or modify dual risk methodology to groundwater concentrations of atrazine and nitrate, or other contaminant mixtures
- Concentrate on "hot spots" to find best places to implement best management practices

E. coli **Project (Objectives 3 and 4)**

- Increase sampling frequency throughout the year, and reduce the total number of post-rainfall samples
- Compare similar sites with and without vegetative filter strips in agricultural settings
- More detailed fall and spring sampling campaigns when migratory waterfowl are present

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